NOVEL COUMARINS, THEIR CARBOXAMIDE DERIVATIVES, PREPARATION METHODS, COMOPOSITIONS, AND USES

TECHNICAL FIELD

The present invention relates to novel coumarins, their carboxamide derivatives, the preparation method thereof and the pharmaceutical compositions containing them, and their use as medicaments for kidney protection, as well as for the treatment of hypertension, cardio- cerebrovascular diseases, non-insulin dependent diabetes (NIDD), tumor, preneoplastic lesions, and edemas.

BACKGROUND ART

In 1990 the German Federal Institute of Drugs and Medicinnal Products (BfArM) published a monograph on *Meliloti Herba*, in which the use of melilot is indicated for symptoms and signs in chronic venous insufficiency like pains; adjuvant treatment of thrombophlebitis and lymphostasis. Scheel et al. (*Microbiol Toxins* 8: 47-66, 1972) reported that coumarins have anti-bacterial, anti-viral and anti-tumor effects. Kovach et al (*Arzeim-Forsch/Drug Res* 20: 1630- 33, 1970) proved that coumarins can increase blood flow and improve myocardial ischemia. Casley-Smith, (*Vasomed* 6: 232-4, 1994), Gaffney (*J Pathol* 133: 229-42, 1981), Piller (*Br J Exp Pathol* 59: 319-26, 1978), and Knight (*Clin Sci* 77: 69-76, 1989) showed that coumarins have effects of endothelial system protection and lymph-circulation promotion, etc. Nair et al. (*Carcinogenesis* **12** (1): 65-69, 1991) reported the anticancer activity of coumarins compounds. Ishizuka et al. (US 5, 096, 924) proved that substituted coumarins have anticancer activities. Marshall et al. (*J. Biol. Resp. Mod.* 8: 62, 1989) reported that coumarins have immuno-enhancing effects, such as improving the antitumor abilities by

remarkably raising monocytes of patients suffering from cancer. Preuss-Ueberschar et al. (Drug Res. 34: 1305-1313, 1984) showed that the coumarins are non-teratogenitic. Takagaki, Hidetsugu et al. (EP 0, 796, 854 A1, 1997) disclosed that 3-, 4-, or 7-hydroxy or alkoxy substituted coumarins' effects in treating heart diseases. Markal et al. (WO 98/25, 608, 1998) disclosed that substituted 4-arylcoumarins can be used to treat viral infections, such as herpes zoster or herpes simplex. Trkovnik et al. (WO 99/21550, 1999) reported that 4-methyl-7-hydroxycoumarin can be used to treat or prevent nephrocirrhosis, pancreatitis, and disorders in bladder or alimentary tracts. Takagaki et al. (Jpn. Kokai Tokkyo Koho JP 07277972, 1995) reported that coumarin derivatives can inhibit rat diabetes induced by streptozotocin. Scott et al. (US 5, 723, 476, 1998) disclosed that 3-carboxamide-4-hydroxy coumarin compounds are effective to the non-insulin dependent diabetes models. Han, Xingmei et al. (*Zhongguo Yaolixue Tongbao*, 15(4): 332-5, 1999) reported that 6, 7-dimethoxycoumarin is effective to acute renal failure induced by endotoxins. Allen et al. (PCT Int Appl WO 2001 019396 A1 2001) reported that the TGF-β1 antagonists may be used for the treatment or prophylaxis of chronic nephritis.

In our research, a series of coumarin derivatives were synthesized and their biological activities were evaluated. For example, Xiao-long Huang et al reported substituted 3-acetyl- and 3-glyoxal-coumarin derivatives possessing good anti-mutagenic effects (*Yaoxue Xuebao* 31(6): 431-436, 1996; ibid 31(7): 509- 516, 1996). Shi-ping Xu et al discovered that the coumarin retinoids show potent differentiation inducing, anti-mutagenic, and anti-carcinogenic effects (Chinese patent application No. 97116602.1, CN1207392A). Song Xu et al.'s study on 6- or 7-substitutedstyryl-coumarins, 4-styryl-coumarins and 4-, 6- or 7-substitutedphenyliminomethylene-coumarins show anticancer effects

(*Yaoxue Xuebao* 35(2): 103-107, 2000; ibid 36(4): 269-273, 2001; ibid 37(2): 113-116, 2002).

Following that, upon our continued research works on coumarins compounds, a series of novel coumarins and coumarin carboxamides were synthesized. And we have found that these coumarins carboxamide compounds possess potent inhibition effects on transforming growth factor β1 (TGF-β1), which has not been reported before. The TGF-β1 inhibitors may be used for the treatment of chronic renal dysfunctions and diabetic renal dysfunctions. Meanwhile, it can also significantly decrease angiotensin II (AngII). Therefore, the compounds of present invention, may be used in the drugs for the treatment of chronic renal failure, nephritis, hypertension, even cirrhosis of liver and pulmonary fibrosis. For example, Allen et al. (PCT Int Appl WO 2001 019396 A1 2001) reported that the TGF-β antagonists may be used for the treatment or prophylaxis of chronic nephritis.

Renal insufficiencies, particularly chronic renal failure, are the results of kidney injuries with various pathogenesis and progressive deterioration. Among the primary nephropathies, the most common is the chronic glomerulonephritis, and tubolointerstitial nephritic comes the second. Among the secondary nephropathies, diabetic nephropathy holds the first position. At present, diabetic nephropathy holds about 27% of the origin of chronic renal failures, and is still increasing; hypertensive nephropathy comes the second, about 22.7% and the glomerulonephritis comes next about 21.2% and all other pathogenesis occupy 26.6% in the origin of chronic renal insufficiencies. Being a common disease per se, nephropathies, no wonder what pathogenesis, or being immune or non-immune mechanism, unless promptly treated, may be result in chronic renal insufficiency and irreversible chronic renal injuries.

Upon the researches of the field, it shows that transforming

growth factor-β1 (TGF-β1) has a close relationship with chronic renal insufficiencies caused by various pathogenesis. Four hours after nephrectomy, TGF-β1 began to increase and the renin-angiotensin system was consequently influenced. Continuously rising of TGF-β1 and over-expression will result in inhibiting the degradation of the extracellular matrix, promoting the matrix integration, and also relates to the proteinuria from renal insufficiency, as well as matrix fibrosis. Therefore, glomerular sclerosis and interstitial fibrosis have direct relationship with TGF-β1, and renin-angiotensin system and TGF-β1 are the two critical factors related to chronic renal insufficiencies. Moreover, as the inhibition of the former has close relationship with the decrease of TGF-β1 producing, this suggests that the increase of TGF-β1 might be an important pathogenesis of kidney injuries to the end-stage renal insufficiencies, and would be a new target for the screening of new anti-renal failure drugs.

Coumarin compounds possess extensive biological activities, however, it haven't been reported in the literatures that these compounds can be used for the treatment of chronic renal failures. The present compounds are a novel type compounds and can remarkably inhibit TGF-β1, which is an important pathogenesis of kidney injuries to the end-stage renal failure. It has been proved that, the compound of present invention show satisfied effects on treating renal insufficiencies.

Renal insufficiencies, especially chronic renal insufficiencies, are chronic diseases that are hard to be cured. With the continuously increase of diabetes and hypertension, the incidence of renal insufficiencies becomes more and more, and has no effective drugs or other methods for the treatment or prophylaxis up to now. Therefore, the object of the present invention is to develop new drug for the treatment of renal insufficiencies.

SUMMARY OF THE INVENTION

An object of the present invention is to overcome one or more deficiencies of the prior arts, and to provide a new coumarin, in particular to provide low toxic carboxamide derivatives thereof.

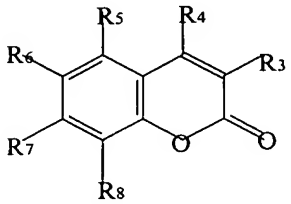
One another object of the present invention is to provide a preparation method of the coumarin carboxamide derivatives.

One aspect of the present invention relates to pharmaceutical compositions, which comprises a compound of general formula (I) or an isomer thereof as the active ingredient, and pharmaceutically acceptable carriers.

A further object of the present invention is to provide use of the novel coumarin carboxamide derivatives or the compositions thereof for the as TGF-β1 and angiotensin II (AngII) inhibitors.

A still further object of the present invention is to provide use of the novel coumarin carboxamide derivatives or the compositions thereof for the preparation of the medicaments for the treatment of kidney disorders (such as various chronic nephritis, diabetic and hypertensive renal insufficiency), non-insulin dependent diabetes, cardio-cerebrovascular diseases and hypertension.

Specifically, the first aspect of the present invention relates to the compounds of the general formula (I)

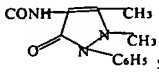


(I)

Wherein,

R^3 is selected from the group consisting of H, carboxyl,

alkyloxycarbonyl, 5'-(phenyloxadiazol-2')-yl,

5'-(pyridyl-4"-oxadizol-2')-yl,  , $CONHR_9$, wherein R_9 is selected from the group consisting of C_2-C_8 fatty acid, benzoxamido, isonicotinamido, un-substituted or mono- or multi-substituted phenyl wherein the substituent may be hydroxyl, C_1-C_8 alkoxyl, CF_3, carboxyl, alkyloxycarbonyl, OCH_2CO_2H, NO_2, halogen, SO_3H, SO_2NHR_{11}, wherein R_{11} is selected from the group consisting of hydrogen, amidino, 2"-thiazolyl, 3"-(5"-methylisooxazolyl), 2"-pyrimidinyl, 2"-(4", 6"-dimethylpyrimidinyl), 4"-(5", 6"-dimethoxypyrimidinyl);

R_4 is selected from the group consisting of hydrogen, $CONHR_{10}$, wherein R_{10} is selected from the group consisting of C_2-C_8 fatty acid, benzoxamido, isonicotiniamido, un-substituted, mono- or multi-substituted phenyl wherein the substituent may be hydroxyl, C_1-C_8 alkoxyl, CF_3, carboxyl, alkoxycarbonyl, OCH_2CO_2H, NO_2, halogen, SO_3H, SO_2NHR_{12}, wherein R_{12} is selected from the group consisting of H, amidino, 2"-thiazolyl, 3"-(5"-methylisooxazolyl), 2"-pyrimidinyl, 2"-(4", 6"-dimethyl- pyrimidinyl), 4"-(5", 6"-dimethoxy pyrimidinyl);

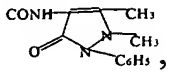
R_5 is selected from the group consisting of H, C_1-C_4 alkyl;

R_6 is selected from the group consisting of H, C_1-C_{12} alkyl, halogen, NO_2, $CONHR_{13}$, wherein R_{13} is substituted phenyl;

R_7 is selected from the group consisting of H, hydroxyl, C_1-C_4 alkyl or alkoxyl, carboxylalkylenoxyl, OCH_2CONHR_{14}, wherein R_{14} is selected from the group consisting of un-substituted, mono- or multi-substituted phenyl wherein the substituent may be hydroxyl, OCH_3, CF_3, CO_2H, $CO_2C_2H_5$, NO_2;

R_8 is selected from the group consisting of H, C_1-C_4 alkyl or alkoxyl, NO_2;

In order to achieve the object of the present invention, preferable

compounds include, but are not limited to the following compounds:
R_3 is selected from the group consisting of H, COOH, $CO_2C_2H_5$, 5'-(phenyloxadiazol-2')-yl, 5'-(pyridyl-4"-oxadizol-2')-yl,  , $CONHR_9$, wherein R_9 is n-butyric acid, o-, m-, p-phenol, o-, m-, p-carboxyl-phenyl, o-, m-, p-alkyloxycarbophenyl, methoxylphenyl, 3'-hydroxy-4'-carboxyphenyl, 3'-salicylyl, 4'-salicylyl, m-CF_3-phenyl, 3'-CF_3-4'-NO_2-phenyl, 2'-CO_2H-4'-I-phenyl, isonicotinamido, benzoxamido, 3'-carboxy-methylenoxyphenyl, 4'-amidosulfonylphenyl, 4'-guanidinosulfonylphenyl, 4'-(2"-thiazolamidosulfonyl)phenyl, 4'-(5"-methylisooxazolyl-3"-amidosulfonyl)phenyl, 4'-(pyrimidinyl-2"-amidosulfonyl)phenyl, 4'-(4", 6"-dimethylpyrimidinyl-2"-amidosulfonyl)phenyl, 4'-(5", 6"-dimethoxypyrimidinyl-4"-amidosulfonyl)phenyl;

R_4 is selected from the group consisting of H, $CONHR_{10}$, wherein R_{10} is selected from the group consisting of H, 4'-CO_2H-phenyl, 4'-$CO_2C_2H_5$phenyl, 3'-CF_3-phenyl;

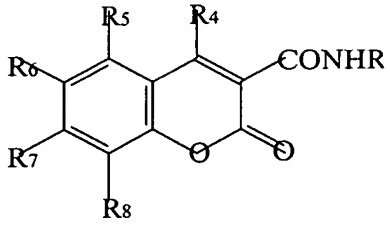
R_5 is selected from the group consisting of H, CH_3;

R_6 is selected from the group consisting of H, C_2H_5, n-C_6H_{13}, NO_2, NH_2, Cl, Br, $CONHR_{13}$, wherein R_{13} is selected from the group consisting of carboxyl- and alkoxycarbonyl- substituted phenyl;

R_7 is selected from the group consisting of H, OH, CH_3, OCH_3, OCH_2CONHR_{14}, wherein R_{14} is selected from the group consisting of phenyl, o-, m- and p-hydroxyphenol, o-, m- and p-carboxylphenyl, m- and p-ethoxycarbonylphenyl, m-CF_3-phenyl, m-CF_3-p-NO_2-phenyl, p-CH_3O-phenyl, 4-salicylyl, 3-salicylyl;
R_8 is selected from the group consisting of H, CH_3, OCH_3, NO_2;

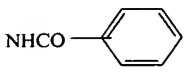
In order to complete the object of the present invention, preferable compounds include, but are not limited to the compounds

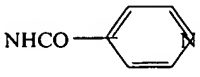
represented by following general formula (Ia):

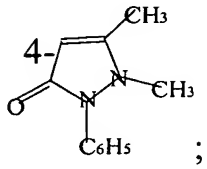


(Ia)

wherein R_4, R_5, R_6, R_7, R_8 are defined same as general formula (I),

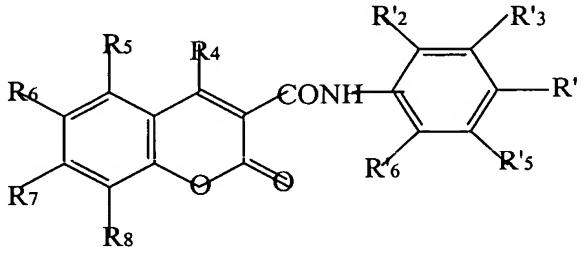
R=$(CH_2)_3CO_2H$, 

, 

, 

;

In order to complete the object of the present invention, preferable compounds include, but are not limited to the compounds represented by the following general formula (Ib):



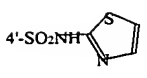
(Ib)

wherein R_4, R_5, R_6, R_7, R_8, are same as defined in general formula (I),

R'$_2$ is selected from the group consisting of H, OH, CO_2H, etc;

R'$_3$ is selected from the group consisting of H, OH, CO_2H, CF_3, OCH_2CO_2H, etc;

R'$_4$ is selected from the group consisting of H, OH, CO_2H, CO_2Et, Iodo, NO_2, OCH_3, SO_3H, SO_2NH_2, $SO_2NH(C=NH)NH_2$,



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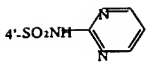

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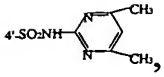

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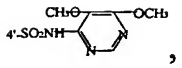

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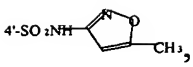


,

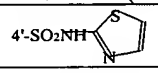
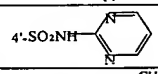
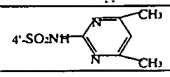
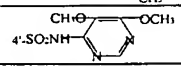
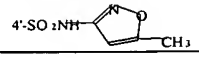
etc;

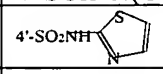
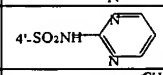
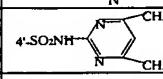
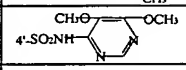
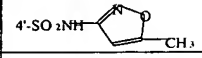
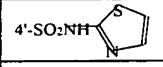
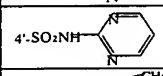
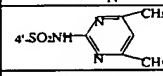
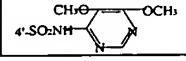
R'_5 and R'_6 each is H;

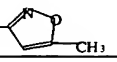
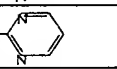
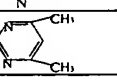
In order to complete the object of the present invention, preferable compounds include, but are not limited to the compounds of the following tables 1 and 2:

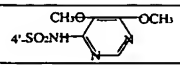
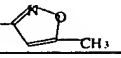
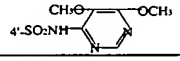
Wherein R_4-R_8 groups are H except specified, R'_2-R'_6 groups are H except specified

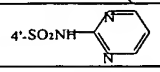
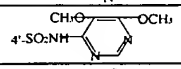
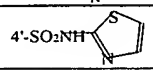
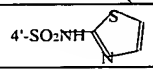
Table 1

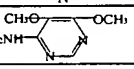
No.	R_4—R_8		MP°C
1	7-OCH_3	4'-COOH	>300
2	7-OCH_3	3'-COOH	>300
3	7-OCH_3	2'-COOH	>300
4	7-OCH_3	2'-OH	>300
5	7-OCH_3	3'-OH	265
6	7-OCH_3	4'-OH	>300
7	7-OCH_3	3'-OH, 4'-COOH	279d
8	7-OCH_3	3'-COOH, 4'-OH	230d
9	7-OCH_3	2'-COOH, 4'-I	>300
10	7-OCH_3	4'-COOEt	247
11	7-OCH_3	3'-CF_3	218
12	7-OCH_3	3'-CF_3 4'-NO_2	>300
13	7-OCH_3	4'-SO_2NH_2	>300
14	7-OCH_3	4'-$SO_2NH(C=NH)NH_2$	>300
15	7-OCH_3		>300
16	7-OCH_3		>300
17	7-OCH_3		298
18	7-OCH_3		300
19	7-OCH_3		282d
20	7-OCH_3	4'-OCH_3	233
21	7-OCH_3	4'-SO_3H	284
22	6-Et 7-OCH_3	4'-COOH	>300
23	6-Et 7-OCH_3	3'-COOH	298
24	6-Et 7-OCH_3	2'-COOH	294
25	6-Et 7-OCH_3	4'-OH	304
26	6-Et 7-OCH_3	3'-OH, 4'-COOH	266

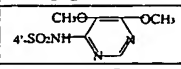
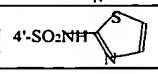
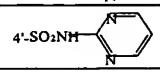
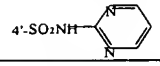
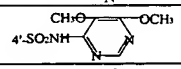
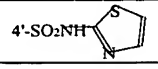
27	6-Et 7-OCH_3	3'-COOH, 4'-OH	298
28	6-Et 7-OCH_3	4'-COOEt	233
29	6-Et 7-OCH_3	3'-CF_3	224
30	6-Et 7-OCH_3	3'-CF_3 4'-NO_2	234
31	6-Et 7-OCH_3	4'-SO_2NH_2	>300
32	6-Et 7-OCH_3	4'-$SO_2NH(C=NH)NH_2$	299
33	6-Et 7-OCH_3		>300
34	6-Et 7-OCH_3		>300
35	6-Et 7-OCH_3		278
36	6-Et 7-OCH_3		260d
37	6-Et 7-OCH_3		>300
38	6-Et 7-OCH_3	4'-OCH_3	202
39	6-Et 7-OCH_3	4'-SO_3H	>300
40	7-OCH_3 8-CH_3	4'-COOH	>300
41	7-OCH_3 8-CH_3	3'-COOH	>300
42	7-OCH_3 8-CH_3	2'-COOH	264
43	7-OCH_3 8-CH_3	3'-OH, 4'-COOH	279
44	7-OCH_3 8-CH_3	3'-COOH, 4'-OH	290
45	7-OCH_3 8-CH_3	2'-COOH, 4'-I	284
46	7-OCH_3 8-CH_3	4'-COOEt	270
47	7-OCH_3 8-CH_3	3'-CF_3	258
48	7-OCH_3 8-CH_3	3'-CF_3, 4'-NO_2	252
49	7-OCH_3 8-CH_3	4'-SO_2NH_2	300
50	7-OCH_3 8-CH_3	4'-$SO_2NH(C=NH)NH_2$	>300
51	7-OCH_3 8-CH_3		>300
52	7-OCH_3 8-CH_3		277
53	7-OCH_3 8-CH_3		220d
54	7-OCH_3 8-CH_3		286

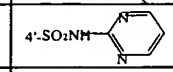
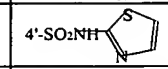
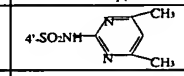
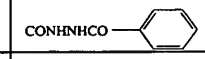
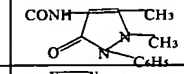
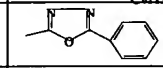
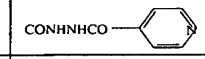
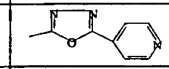
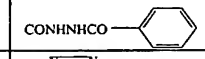
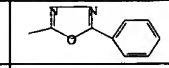
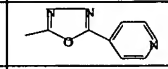
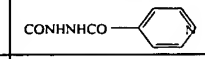
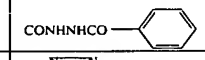
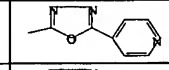
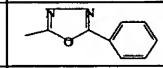
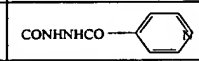
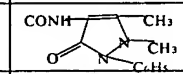
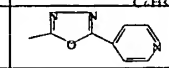
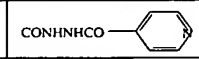
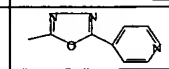
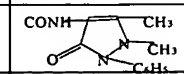
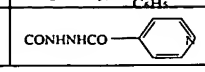
55	7-OCH_3 8-CH_3		286
56	7-OCH_3 8-CH_3	4'-OCH_3	258
57	7-OCH_3 8-CH_3	4'-SO_3H	286
58	7-OCH_3 8-OCH_3	4'-COOH	315
59	7-OCH_3 8-OCH_3	3'-OH, 4'-COOH	264
60	7-OCH_3 8-OCH_3	3'-COOH, 4'-OH	264
61	7-OCH_3 8-OCH_3	4'-COOEt	236
62	7-OCH_3 8-OCH_3	3'-CF_3	243
63	7-OCH_3 8-OCH_3	3'-CF_3, 4'-NO_2	283
64	7-OCH_3 8-OCH_3	3'-OCH_2COOH	188
65	7-OCH_3 8-OCH_3	4'-SO_2NH_2	280
66	7-OCH_3 8-OCH_3	4'-$SO_2NH(C=NH)NH_2$	252
67	5-CH_3 7-OCH_3	4'-COOH	299
68	5-CH_3 7-OCH_3	3'-COOH	>300
69	5-CH_3 7-OCH_3	2'-COOH	>300
70	5-CH_3 7-OCH_3	2'-OH	246
71	5-CH_3 7-OCH_3	3'-OH	292
72	5-CH_3 7-OCH_3	4'-OH	255
73	5-CH_3 7-OCH_3	3'-OH, 4'-COOH	284
74	5-CH_3 7-OCH_3	3'-COOH 4'-OH	>300
75	5-CH_3 7-OCH_3	4'-COOEt	265
76	5-CH_3 7-OCH_3	3'-CF_3	221
77	5-CH_3 7-OCH_3	3'-CF_3, 4'-NO_2	>300
78	5-CH_3 7-OCH_3	4'-SO_2NH_2	283
79	5-CH_3 7-OCH_3	4'-$SO_2NH(C=NH)NH_2$	293
80	5-CH_3 7-OCH_3		288
81	5-CH_3 7-OCH_3		>300
82	5-CH_3 7-OCH_3		274d

83	5-CH_3 7-OCH_3		268
84	5-CH_3 7-OCH_3		271
85	5-CH_3 7-OCH_3	4'-OCH_3	210
86	6-Cl 7-OCH_3	4'-COOH	>300
87	6-Cl 7-OCH_3	3'-OH, 4'-COOH	253
88	6-Cl 7-OCH_3	3'-COOH, 4'-OH	>300
89	6-Cl 7-OCH_3	4'-COOEt	294
90	6-Cl 7-OCH_3	3'-CF_3	282
91	6-Cl 7-OCH_3	4'-SO_2NH_2	>300
92	6-Cl 7-OCH_3	4'-$SO_2NH(C=NH)NH_2$	302
93	6-Cl 7-OCH_3		317
94	6-Br 7-OCH_3	4'-COOH	>300
95	6-Br 7-OCH_3	2'-COOH	288
96	6-Br 7-OCH_3	3'-OH, 4'-COOH	298
97	6-Br 7-OCH_3	2'-COOH, 4'-I	>300
98	6-Br 7-OCH_3	4'-COOEt	298
99	6-Br 7-OCH_3	3'-CF_3	284
100	6-Br 7-OCH_3	4'-SO_2NH_2	298
101	6-Br 7-OCH_3	4'- OCH_3	262
102	6-nHex 7-OCH_3	4'-COOH	248
103	6-nHex 7-OCH_3	2'-COOH	272
104	6-nHex 7-OCH_3	3'-OH, 4'-COOH	268
105	6-nHex 7-OCH_3	2'-COOH, 4'-I	249
106	6-nHex 7-OCH_3	4'-COOEt	160
107	6-nHex 7-OCH_3	3'-CF_3	201
108	6-nHex 7-OCH_3	4'-SO_2NH_2	242
109	6-nHex 7-OCH_3	4'- OCH_3	170
110	6-NO_2 7-OCH_3 8-OCH_3	4'-COOH	>300
111	6-NO_2 7-OCH_3 8-OCH_3	3'-COOH	232

112	6-NO_2 7-OCH_3 8-OCH_3	4'-OCH_3	256
113	6-NO_2 7-OCH_3 8-OCH_3	3'-OH	160
114	6-NO_2 7-OCH_3 8-OCH_3	2'-OH	217
115	6-NO_2 7-OCH_3 8-OCH_3	4'-COOEt	193
116	6-NO_2 7-OCH_3 8-OCH_3	3'-OH, 4'-COOH	>300
117	6-NO_2 7-OCH_3 8-OCH_3	3'-COOH, 4'-OH	266d
118	6-NO_2 7-OCH_3 8-OCH_3	3'-CF_3	218
119	6-NO_2 7-OCH_3 8-OCH_3	3'-CF_3, 4'-NO_2	217
120	6-NO_2 7-OCH_3 8-OCH_3	4'-SO_2NH_2	290d
121	6-NO_2 7-OCH_3 8-OCH_3	4'-$SO_2NH(C=NH)NH_2$	284
122	6-NO_2 7-OCH_3 8-OCH_3		190d
123	6-NO_2 7-OCH_3 8-OCH_3		220d
124	6-NO_2 7-OCH_3 8-OCH_3		200d
125	6-C_2H_5, 7-OH 8-NO_2	4'-COOH	234
126	6-C_2H_5, 7-OH 8-NO_2	4'-OCH_3	218d
127	6-C_2H_5, 7-OH 8-NO_2	3'-OH	>300
128	6-C_2H_5, 7-OH 8-NO_2	2'-OH	248d
129	6-C_2H_5, 7-OH 8-NO_2	4'-COOEt	160
130	6-C_2H_5, 7-OH 8-NO_2	3'-OH, 4'-COOH	>300
131	6-C_2H_5, 7-OH 8-NO_2	3'-COOH, 4'-OH	>300
132	6-C_2H_5, 7-OH 8-NO_2	3'-CF_3	169
133	6-C_2H_5, 7-OH 8-NO_2	4'-SO_2NH_2	206d
134	6-C_2H_5, 7-OH 8-NO_2	4'-$SO_2NH(C=NH)NH_2$	181
135	6-C_2H_5, 7-OH 8-NO_2		>300
136	6-C_2H_5 7-OCH_3 8-NO_2	4'-COOH	273
137	6-C_2H_5 7-OCH_3 8-NO_2	4'-OH	252
138	6-C_2H_5 7-OCH_3 8-NO_2	4'-OCH_3	169
139	6-C_2H_5 7-OCH_3 8-NO_2	4'-COOEt	186
140	6-C_2H_5 7-OCH_3 8-NO_2	4'-$SO_2NH(C=NH)NH_2$	206d

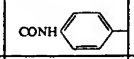
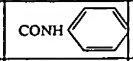
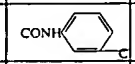
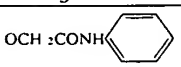
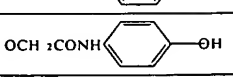
141	6- NO_2, 7-OH, 8-CH_3	4'-COOH	>300
142	6- NO_2 , 7-OH, 8-CH_3	2'-COOH	227
143	6- NO_2 , 7-OH, 8-CH_3	4'-OH	>300
144	6- NO_2 , 7-OH, 8-CH_3	3'-OH	>300
145	6- NO_2 , 7-OH, 8-CH_3	2'-OH	>300
146	6- NO_2 , 7-OH, 8-CH_3	4'-OCH_3	254
147	6- NO_2 , 7-OH, 8-CH_3	4'-COOEt	>300
148	6- NO_2 , 7-OH, 8-CH_3	3'-OH, 4'-COOH	271
149	6- NO_2 , 7-OH, 8-CH_3	3'-COOH, 4'-OH	>300
150	6- NO_2 , 7-OH, 8-CH_3	3'- CF_3	231
151	6- NO_2 , 7-OH, 8-CH_3	3'- CF_3 , 4'-NO_2	226
152	6- NO_2 , 7-OH, 8-CH_3	4'-SO_2NH_2	>300
153	6- NO_2 , 7-OH, 8-CH_3	4'-$SO_2NH(C=NH)NH_2$	>300
154	6- NO_2 , 7-OH, 8-CH_3		>300
155	6- NO_2, 7-OH, 8-CH_3		254
156	6- NO_2, 7-OH, 8-CH_3		>300
157	6- NO_2 , 7-OH, 8-CH_3	2'-COOH, 4'-I	262
158	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-COOH	301
159	6- NO_2 , 7-OCH_3 , 8-CH_3	3'-COOH	280
160	6- NO_2 7-OCH_3 , 8-CH_3	2'-COOH	282
161	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-OH	>300
162	6- NO_2 , 7-OCH_3 , 8-CH_3	3'-OH	231
163	6- NO_2 , 7-OCH_3 , 8-CH_3	2'-OH	285
164	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-OCH_3	209
165	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-COOEt	230
166	6- NO_2 , 7-OCH_3 , 8-CH_3	3'-OH, 4'-COOH	249
167	6- NO_2 , 7-OCH_3 , 8-CH_3	3'-CF_3	182
168	6- NO_2 , 7-OCH_3 , 8-CH_3	3'-CF_3, 4'-NO_2	236
169	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-$SO_2NH(C=NH)NH_2$	>300

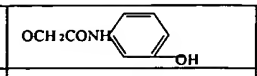
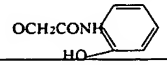
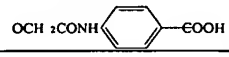
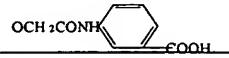
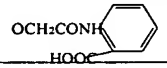
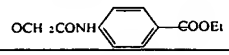
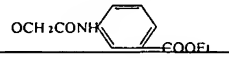
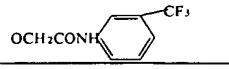
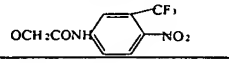
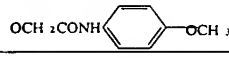
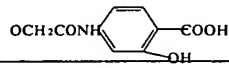
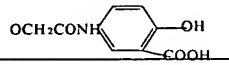
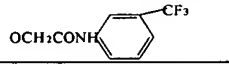
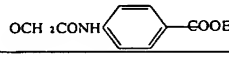
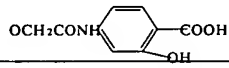
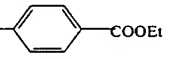
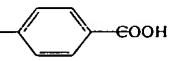
170	6- NO_2 , 7-OCH_3 , 8-CH_3	4'-SO_2NH_2	301
171	6- NO_2 , 7-OCH_3 , 8-CH_3		276
172	6- NO_2 , 7-OCH_3 , 8-CH_3		270
173	6- NO_2 , 7-OCH_3 , 8-CH_3		299
174	6-NO_2 , 7-OH, 8- NO_2	4'-COOH	>300
175	6-NO_2 , 7-OH, 8- NO_2	4'-OH	260
176	6-NO_2 , 7-OH, 8- NO_2	3'-OH	>300
177	6-NO_2 , 7-OH, 8- NO_2	2'-OH	>300
178	6-NO_2 , 7-OH, 8- NO_2	4'-OCH_3	>300
179	6-NO_2 , 7-OH, 8- NO_2	4'-COOEt	281
180	6-NO_2 , 7-OH, 8- NO_2	3'-CF_3	197
181	6-NO_2 , 7-OH, 8- NO_2	4'-SO_2NH_2	>300
182	6-NO_2 , 7-OH, 8- NO_2	4'-$SO_2NH(C=NH)NH_2$	216
183	6-NO_2 , 7-OH, 8- NO_2		>300
184	6-NO_2 , 7-OH, 8- NO_2		170
185	6-NO_2 , 7-OH, 8- NO_2		>300
186	6-NO_2 , 7-OH, 8- NO_2	2'-COOH	285
187	6-NO_2 , 7-OCH_3 8- NO_2	4'-OH	257
188	6-NO_2 , 7-OCH_3 8- NO_2	4'-COOEt	236
189	6-NO_2 , 7-OCH_3 8- NO_2	4'-OCH_3	205
190	6-Cl 7- OH 8- NO_2	4'-OCH_3	285
191	6-Cl 7- OH 8- NO_2	4'-$SO_2NH(C=NH)NH_2$	300d
192	6-Cl 7- OH 8- NO_2	3'-OH 4'-COOH	>300
193	5-CH_3, 6-, 8-$(NO_2)_2$ 7- OH	4'-COOH	>300
194	5-$CH_3$6-, 8- $(NO_2)_2$, 7- OH	3'-COOH	246
195	5-CH_3 6-, 8- $(NO_2)_2$7- OH	2'-COOH	214
196	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH	4'-OCH_3	242
197	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH	4'-COOEt	244

198	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH	4'-SO_2NH_2	256
199	5-CH_3, 6-, 8-$(NO_2)_2$ 7- OH	4'-$SO_2NH(C{=}NH)NH_2$	>300
200	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH		>300
201	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH		220
202	5-$CH_3$6-, 8- $(NO_2)_2$ 7- OH		276
	R_4-R_8	R_3	
203	7-OCH_3	$CONH(CH)_3CO_2H$	193
204	7-OCH_3		293
205	7-OCH_3		248
206	7-OCH_3		238
207	6-C_2H_5, 7-OCH_3	$CONH(CH)_3CO_2H$	226
208	6-C_2H_5, 7-OCH_3		293
209	6-C_2H_5, 7-OCH_3		196
210	5-CH_3, 7-OCH_3		248
211	5-CH_3, 7-OCH_3		176
212	5-CH_3, 7-OCH_3		240
213	7-OCH_3, 5-CH_3		254
214	7-OCH_3, 8-CH_3		254
215	7-OCH_3 8-CH_3		278
216	7-OCH_3 8-CH_3		270
217	6-Br 7-OCH_3		248
218	6-Br 7-OCH_3		>300
219	6-Br 7-OCH_3		295
220	6-n-C_6H_{13} 7-OCH_3		198
221	6-n-C_6H_{13} 7-OCH_3		196
222	6-n-C_6H_{13} 7-OCH_3		139
223	6-NO_2, 7-OH, 8-CH_3		>300

224	6-NO_2, 7-OCH_3, 8-CH_3	CONHNHCO— 	220
225	6-NO_2 7,8-$(OCH_3)_2$	CONHNHCO— 	199
226	6-NO_2 7;8-$(OCH_3)_2$	CONHNHCO— 	>300

Table 2

No.	R_3	R_4	R_5	R_6	R_7	R_8	MP°C
227	$CO_2C_2H_5$	H	H	NO_2	OCH_3	OCH_3	191
228	CO_2H	H	H	NO_2	OCH_3	OCH_3	188
229	$CO_2C_2H_5$	H	H	NO_2	OH	CH_3	210
230	CO_2H	H	H	NO_2	OH	CH_3	251
231	$CO_2C_2H_5$	H	H	NH_2	OH	CH_3	203
232	CO_2H	H	H	NO_2	OCH_3	CH_3	178
233	$CO_2C_2H_5$	H	H	C_2H_5	OH	NO_2	143
234	CO_2H	H	H	C_2H_5	OH	NO_2	178
235	$CO_2C_2H_5$	H	H	C_2H_5	OCH_3	NO_2	140
236	CO_2H	H	H	C_2H_5	OCH_3	NO_2	176
237	$CO_2C_2H_5$	H	H	NO_2	OH	NO_2	176
238	CO_2H	H	H	NO_2	OH	NO_2	296
239	$CO_2C_2H_5$	H	H	NO_2	OCH_3	NO_2	152
240	CO_2H	H	H	NO_2	OCH_3	NO_2	246
241	$CO_2C_2H_5$	H	H	Cl	OH	NO_2	195
242	CO_2H	H	H	Cl	OH	NO_2	>300
243	CO_2H	H	CH_3	NO_2	OH	NO_2	211
244	$CO_2C_2H_5$	H	CH_3	NO_2	OH	NO_2	104
245	H		H	H	CH_3	H	223
246	H		H	H	CH_3	H	>300
247	H		H	H	CH_3	H	197
248	H	CH_3	H	H		H	230
249	H	CH_3	H	H		H	220

250	H	CH_3	H	H		H	240
251	H	CH_3	H	H		H	196
252	H	CH_3	H	H		H	304
253	H	CH_3	H	H		H	>300
254	H	CH_3	H	H		H	296
255	H	CH_3	H	H		H	207
256	H	CH_3	H	H		H	157
257	H	CH_3	H	H		H	243
258	H	CH_3	H	H		H	301
259	H	CH_3	H	H		H	180
260	H	CH_3	H	H		H	215
261	H	CH_3	H	H		H	277
262	H	CH_3	H	H		CH_3	216
263	H	CH_3	H	H		CH_3	205
264	H	CH_3	H	H		CH_3	260
265	R_3 =R_4= R_5=R_7=R_8=H				R_6= 		214
266	R_3 =R_4= R_5=R_7=R_8=H				R_6= 		300

In this description, the term "halogen" indicates Fluoro, Chloro, Bromo and Iodo. The terms "lower alkane", "lower alkyl" mean linear or branched alkanes and alkyls having 1-6 carbon atoms.

According to the present invention, the compounds may have isomers, generally the said "compounds of the present invention" includes isomers thereof.

The compounds of the present invention may contain cis-/trans-isomers of a double bond, asymmetric center with S- and R-configurations, and include all the potential stereoscopic isomers and

mixtures of two or more isomers. In case that cis-/trans-isomers exist, the present invention also relates to the cis- and trans-isomer and their mixtures, and a pure isomer may be separated according to the conventional methods or synthesized from stereo-selective reagents if necessary.

According to the embodiments of present invention, said compounds may also include the pharmaceutically acceptable salts and hydrate(s), esters, or pro-drugs thereof.

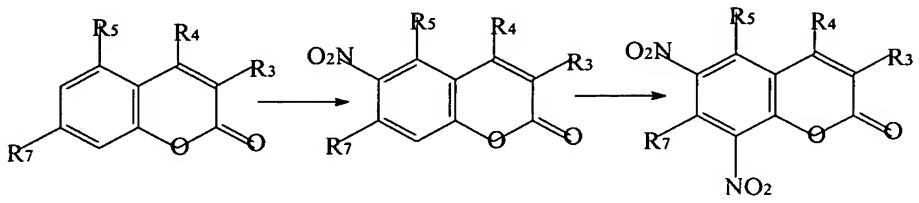
According to the present invention, it is also related to the preparation methods of the compounds of the present invention, which are prepared via nitrating or bi-nitrating various substituted 3-esteryl or 3-carboxy-coumarins, thus to obtain part of the target compounds of present invention and meanwhile the intermediates for the target compounds; reacting the intermediated acids, 3-carboxy-substituted various substituted coumarins, 4-carboxy-substituted various substituted coumarins, 6-carboxy-substituted various substituted coumarins and 7-carboxy-substituted various substituted coumarins with corresponding substituted amines to achieve the target compounds.

The amidating reaction is carried out with corresponding reactants and catalysts, and in the suitable solvents. Said reactants include phosphorus trichloride, phosphorus oxychloride, phosphorus pentachloride, thionyl chloride, 1, 3-dicyclohexylcarbodiimide, dipyridylcarbonate (2-DPC), 1, 3-diisopropylcarbodiimide (DIPC), and 1-(3-dimethylamino-propyl)- 3-ethylcarbodiimide (EDCI), etc. Preferable reactants are phosphorus pentachloride, phosphorus trichloride and thionyl chloride, more preferable reactants are phosphorus pentachloride and thionyl chloride. Catalytic agents for the preparation of the compounds of present invention include tertiary amines, pyridine, 4-dimethylaminopyridine and 4-pyrrolidylpyridine,

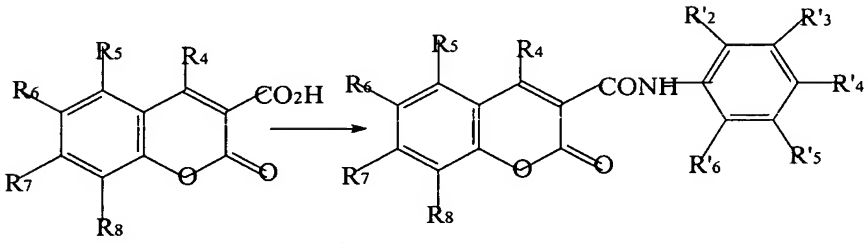
preferably tertiary amines and pyridine, more preferably pyridine. The reaction is carried out in a suitable solvent or the above condensation agent, such as anhydrous aprotic solvent, dimethylsulfoxide (DMSO), toluene, dichloromethane, 1, 2-dichloroethane, ethylene glycol dimethyl ether, tetrahydrofuran and N, N-dimethylformamide (DMF), preferably toluene, DMSO and DMF, more preferably toluene and DMF.

The reaction temperature is 10~110 °C, preferably 20~90 °C, more preferably 30~80 °C, particularly preferably 50~70 °C.

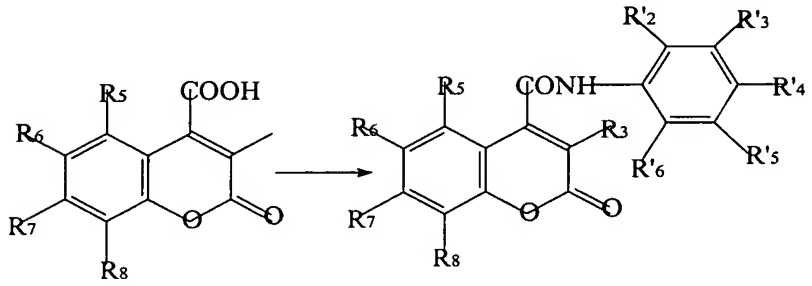
The synthetic routes are specifically explained in the following routes IIa, IIb, IIc, IId, IIe and IIf.



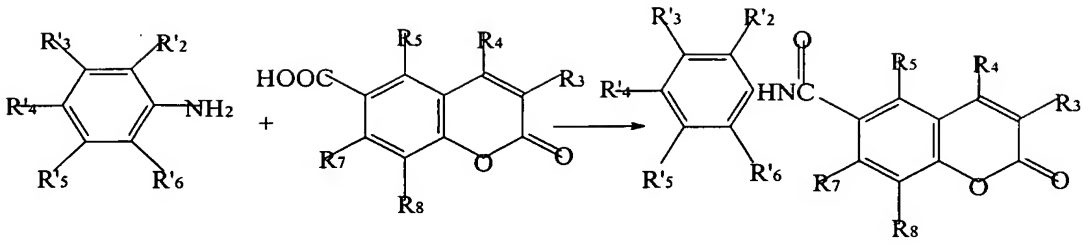
(IIa)



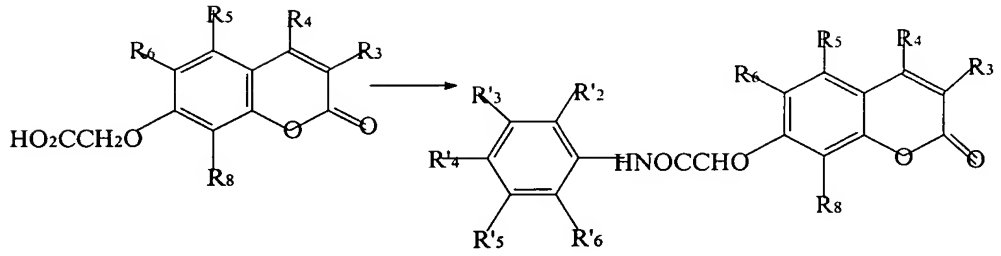
(IIb)



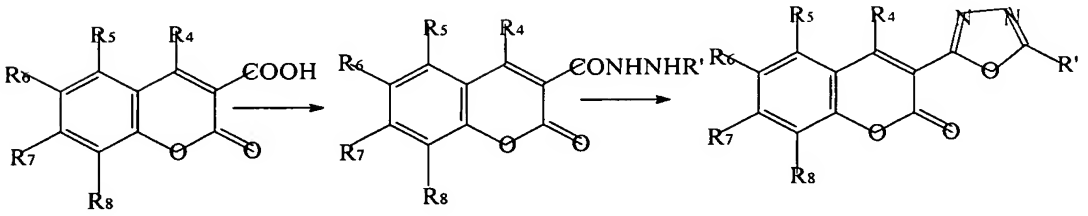
(IIc)



(IId)



(IIe)



(IIf)

Thus, the present invention also relates to a pharmaceutical composition containing a compound of present invention as active ingredient and conventionally pharmaceutical excipients or auxiliaries. Generally the composition may comprise the compound of present invention from 0.1 to 95% by weight.

The composition of present invention may be prepared by the common methods according to the art. For this purpose, the compounds of the present invention may be combined with one or more solid, semi-solid or liquid excipients and/or auxiliaries, to prepare a suitable administration form or dosage forms for human or animal use.

The compounds of the present invention or compositions

containing them may be administered in the unit dosage forms and the administration routes, which can be oral or parenteral, such as oral, intramusculary, subcutaneously, nasal, oral mucosa, transdermal, intraperitoneal or rectal, etc.

The compounds of the present invention or compositions containing them may be administered via injection, including intravenous, intramuscular, subcutaneous, intradermal, and acupoint etc.

The administration forms may be liquid or solid forms. For example the liquid forms may be solutions, colloids, fine particles, emulsion, suspensions and the like. Other forms may be such as tablets, capsules, sprays, dripping pills, aerosols, pills, powders, solutions, suspensions, emulsions, granules, suppositorys, freeze-dried powder for injections and the like.

The present compounds can be prepared as normal preparations, or sustained-release, controlled-release or targeted preparations and various fine particle delivery systems.

In order to prepare unit administration forms to tablets, carriers well known in the art can be used. Carriers such as, diluents and absorbents, such as starch, dextrin, calcium sulfate, lactose, mannitol, sucrose, sodium chloride, glucose, urea, calcium carbonate, kaolin, microcrystallinecellulose, aluminum silicate and the like; moist and binding agents for example water, glycerol, polyethyleneglycol, ethanol, propanol, starch paste, dextrin, syrups, honey, glucose solution, Arabia gum, gelatin, sodium carboxymethylcelluloses, lacta, methylcellulose, potassium phosphate, polyvinylpyrrolidone and the like; disintegrants, for example dry starch, alginates, agar powders, laminarin, sodium hydrogencarbonate-citric acid, calcium carbonate, polyoxyethylene-sorbitol fatty acid esters, sodium lauryl sulfonate, methylcellulose, ethylcellulose and the like; disintegrant inhibitors, such as sucrose,

glycerol tristearate, cocoa butter, hydrogenated oil and the like; absorb-promotors, for example quaternary ammonium salts, sodium lauryl sulfate and the like; lubricants, for example talc, silica, corn starch, stearates, boric acid, liquid paraffin, polyethyleneglycol and the like. The tablets may be further coated, for example sugarcoating, film coating, enteric coating, or two or multi-layered tablets.

To prepare pills from the administration units, carriers well known in the art can be used. Carriers such as, diluents and absorbents, such as glucose, lactose, starch, cocoa butter, hydrogenated vegetable oils, polyvinylpyrrolidone, Gelucire, kaolin, talc and the like; binding agents such as arabia gum, tragacanth, gelatin, ethanol, honey, liquid-sugars, rice pastes or flour pastes and the like; disintegrants such as agar powder, dry starch, alginates, sodium lauryl sulfonate, methylcellulose, ethylcellulose and etc.

To prepare capsules from the administration units, the compounds of present invention may be mixed with the above carrier(s), and the so obtained mixtures are packaged into hard or soft capsules. Alternatively, the present compounds may also be prepared into microcapsules, and can be used as suspension in a hydrous media, or packaged into hard capsules or injections.

To prepare the injection dosage forms, the compounds of present invention may be formulated into solutions, suspensions, emulsions, freeze dried powders for injections. Such formulations may be hydrous or anhydrous, which may contain one or two or more pharmaceutically acceptable carrier(s), diluents, preservatives, surfactants or dispersing agents. For example, diluents are selected from water, ethanol, polyethylene glycol, 1, 3-propylene glycol, ethoxyisostearyl alcohol, polyoxyisostearyl alcohol, polyoxyethylene-sorbitol fatty acid esters and the like. In addition, to prepare the isotonic injections, sodium chloride,

glucose or glycerol can be added to the injection solution, further, solubilizing agents, buffering agents, pH-modulators and the like can also be added.

Also, if desired, coloring agents, preservatives, perfumes, correctants, sweetening agents and the like, can also be added to the pharmaceutical preparation.

For achieving administering purpose and enhancing treating effect, the compounds or pharmaceutical compositions of the present invention may be administered by any known methods. The administering dosage of the present invention may extensively be varied by depending on a number of factors, for example the seriousness of the diseases to be treated or prevented, sex, age, body weights, disposition and individual differences of the patients or animals, administering routes or number of times, treating purposes, etc. In general, the effective dosages of the pharmaceutically active ingredients are known to those skilled in the art, the real administering dose can be suitably adjusted based on the exact dosage contained in the formulations to achieve the treatment or prophylaxis purposes.

The daily suitable ranges of dosages of the compounds of present invention are within 0.001-150mg/kg body weight, preferably 0.1-100mg/kg, more preferably 1-60mg/kg, most preferably 2-30mg/kg. These doses can be administered in one single dose or divided into several doses, for example twice, three or four times per day, which depends on the experiences of doctors and the other different therapeutic means.

The total dose of each treatment may be administered in one portion or divided into multi portions, depending on the total dose. The compound or the pharmaceutical compositions of present invention may be adopted alone or in combination with other drugs, in the latter case,

the dose may also be adjusted.

The activities and effects of the compounds and/or compositions of the present invention may be determined via *in vitro* and *in vivo* tests, such as TGF-β1 antagonism, treating of the renal insufficiencies and the like, which are all known in the filed. In recent years, researches have confirmed that TGF-β1 is one of most critical factors resulting in the progressive renal failure with glomerulosclerosis and interstitial fibrosis.

Pharmacological tests show that the compounds of the present invention possess effects of blocking the binding of TGF-β1 with the receptors and inhibiting the production of TGF-β1. Of all the 33 subject compounds in 10μg/ml doses, 11 compounds possess activities of exceeding 50%, 8 compounds possess activities of exceeding 60%, 7 compounds possess activities of exceeding 70%, 5 compounds possess activities of exceeding 80%, and 4 compounds possess activities of exceeding 90%.

In the cell growth inhibition model of mink pulmonary epithelial cells induced by TGF-β1, of all the 5 subject compounds, 3 compounds possess activities of exceeding 60%, 2 compounds possess activities of exceeding 70%, and 1 compound possesses activity of exceeding 90%. Thus, the present compounds can be used for the treatment or prophylaxis of chronic renal disorders, including: a) primary nephropathies, commonly such as, the chronic glomerulonephritis, interstitial nephritis, chronic pyelonephritis and the like; b) secondary nephropathies, commonly such as, chronic diabetic nephropathy, hypertensive nephropathy, lupus nephropathy and the like; c) congenital and obstructive diseases such as polycystic kidney, posterior urethral valve disorders, neurogenic bladder hyperplasia, prostatic hyperplasia, urinary lithiasis, etc.

Additional researches show that the compounds of the present

invention can remarkably inhibit the effect of Ang II ($P<0.01$). As mentioned above, TGF-β1 and the renin-angiotesin system are closely related with renal insufficiencies having multi pathogenesis. TGF-β1 and the renin-angiotesin system are the two most critical factors in the progressive deterioration of renal disorders and the inhibition of Ang II has a close relationship with the reduction of TGF-β1. As Ang II plays an important role in the onset of various types of hypertensions, the compounds of the present invention may be used for the treatment of renal hypertensions, diabetic hypertensions, peripheral vascular hypertensions and cardio-cerebrovascular diseases having the above pathogenesis.

In vivo tests on the model of chronic renal failure induced by a 5/6 nephrectomy in rats showes that, comparing with the positive controls Benazepril and Losartan, the compounds of the present invention superiors than Benazepril and corresponds to (slightly better than) Losartan on reducing the blood serum urea nitrogen (BUN) and creatinine (Cre), as well as inhibiting TGF-β1 and Ang II.

In the tests of the renal interstitial fibrosis model from unilateral ureteral ligation in rats, the subject compound **149** is better than Benazepril and corresponds to (slightly better than) Losartan, and the pathological results show that the compound of present invention are better than Benazepril and correspond to Losartan.

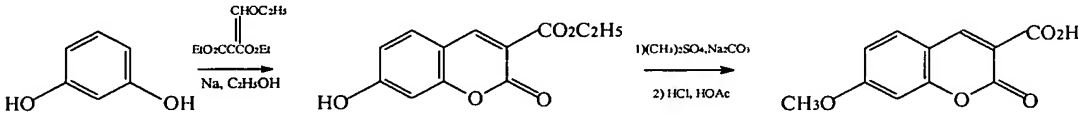
The tested compounds have low toxicities, under the dosages of 5g/kg body weights and 10g/kg body weights, within a continuous observation of two weeks, no death in the subjected mice were observed and no other abnormal expressions were found.

In Ames test of the subjected compound **149,** the negative results was obtained which shows no mutagenesis.

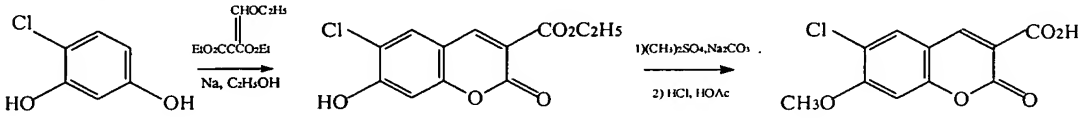
Examples

The various starting materials of the examples can be prepared via the ordinary method of the field or the methods commonly known by the skilled artisans, which can be prepared via e.g. the following reaction routes.

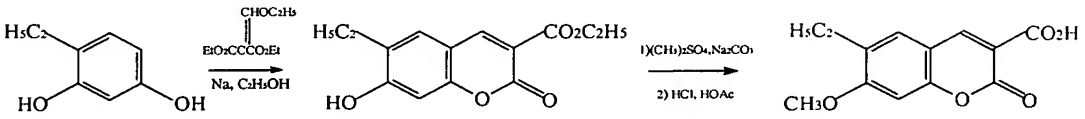
(1) 3-ethoxycarbonyl-7-hydroxycoumarin and 3-carboxy-7-methoxycoumarin



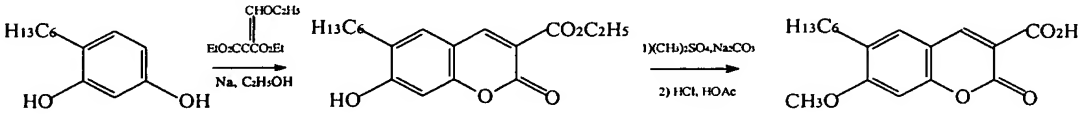
(2) 3-ethoxycarbonyl-6-chloro-7-hydroxycoumarin and 3-carboxy-6-chloro-7-methoxycoumarin



(3) 3-ethoxycarbonyl-6-ethyl-7-hydroxycoumarin and 3-carboxy-6-ethyl-7-methoxycoumarin

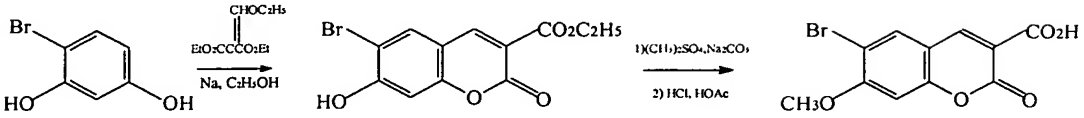


(4) 3-ethoxycarbonyl-6-hexyl-7-hydroxycoumarin and 3-carboxy-6-hexyl-7-methoxycoumarin

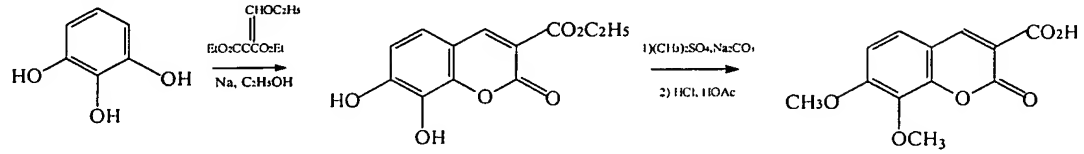


(5) 3-ethoxycarbonyl-6-bromo-7-hydroxycoumarin and

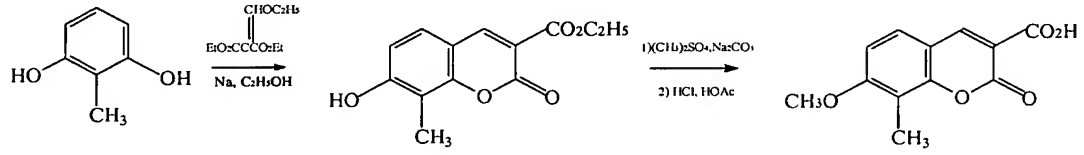
3-carboxy-6-bromo-7-methoxycoumarin



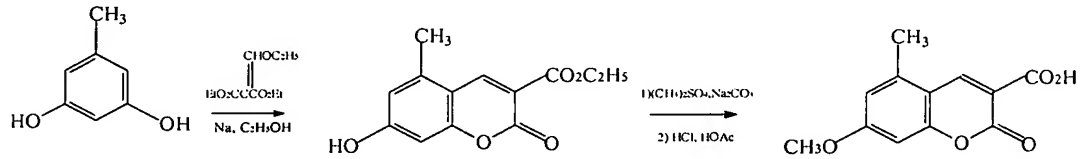
(6) 3-ethoxycarbonyl-7,8-dihydroxycoumarin and 3-carboxy-7,8-dimethoxycoumarin



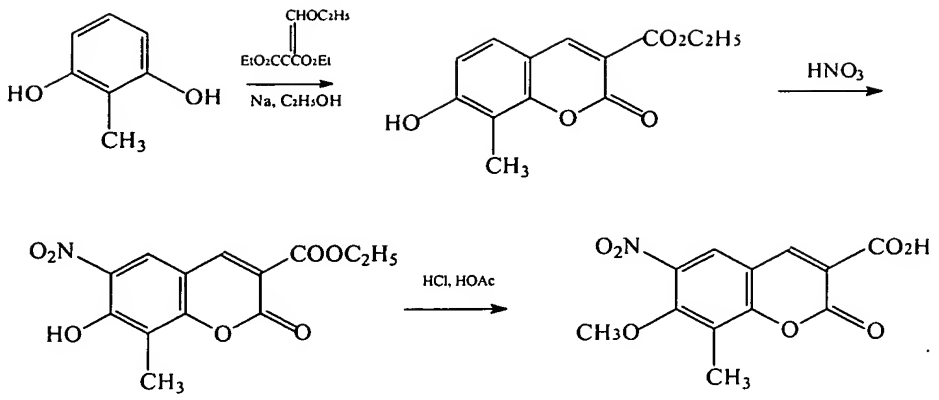
(7) 3-ethoxycarbonyl-7-hydroxy-8-methylcoumarin and 3-carboxy-7-methoxy -8-methylcoumarin



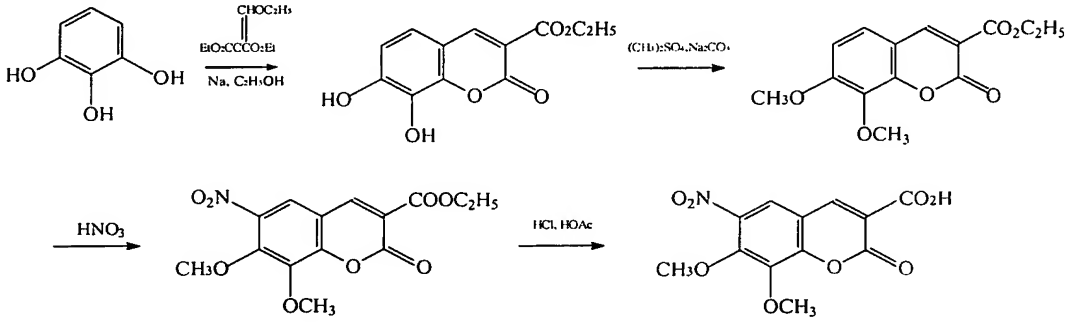
(8) 3-ethoxycarbonyl-7-hydroxy-5-methylcoumarin and 3-carboxy-7-methoxy-5-methylcoumarin



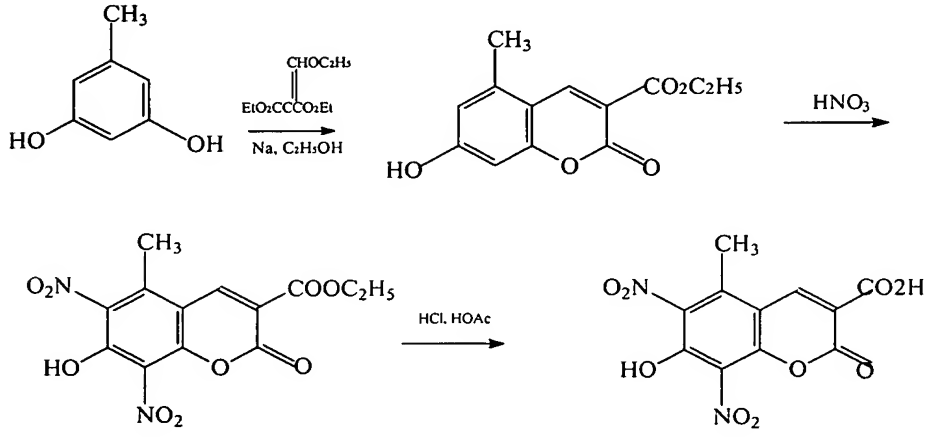
(9) 3-ethoxycarbonyl-6-nitro-7-hydroxy-8-methylcoumarin and 3-carboxy-6-nitro-7-methoxy-8-methylcoumarin



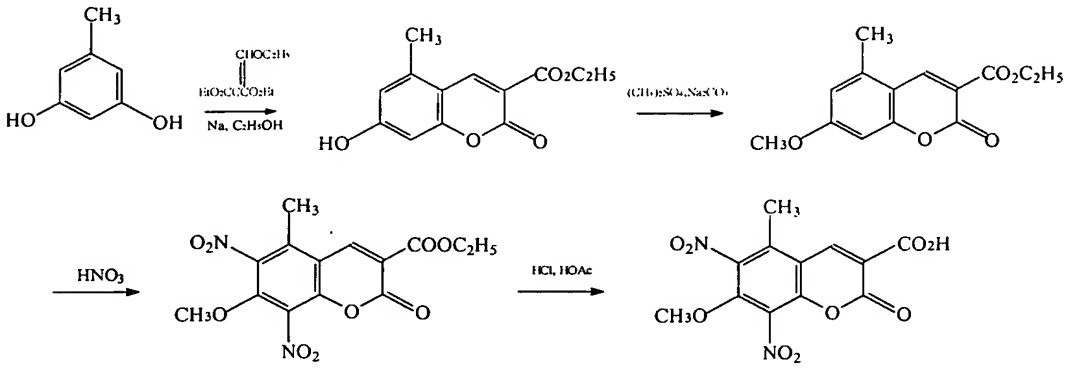
(10) 3-ethoxycarbonyl-6-nitro-7,8-dihydroxycoumarin and 3-carboxy-6-nitro-7,8-dimethoxycoumarin



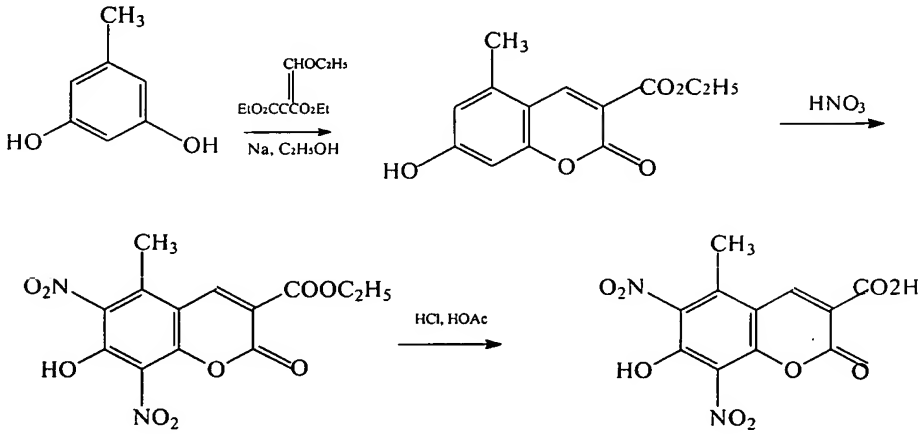
(11) 3-ethoxycarbonyl-5-methyl-6, 8-dinitro-7-hydroxycoumarin and 3-carboxy-5-methyl-6, 8-dinitro-7-hydroxy-coumarin



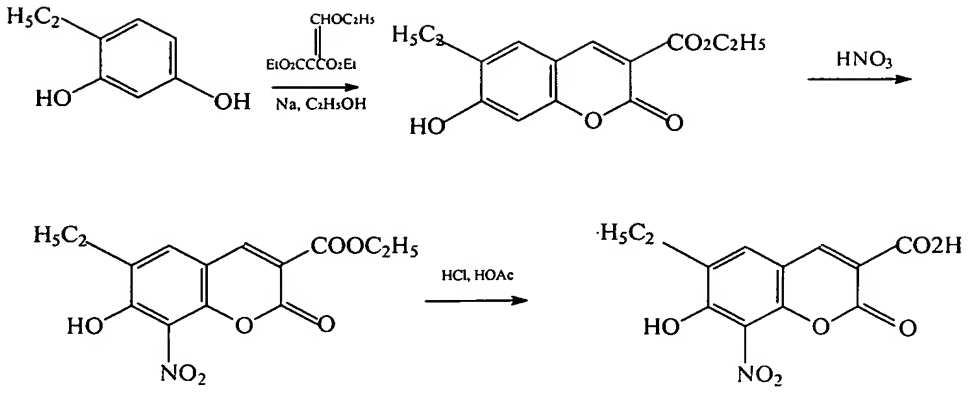
(12) 3-ethoxycarbonyl-5-methyl-6, 8-dinitro-7-methoxycoumarin and 3-carboxy-5-methyl-6, 8-dinitro-7-methoxy-coumarin



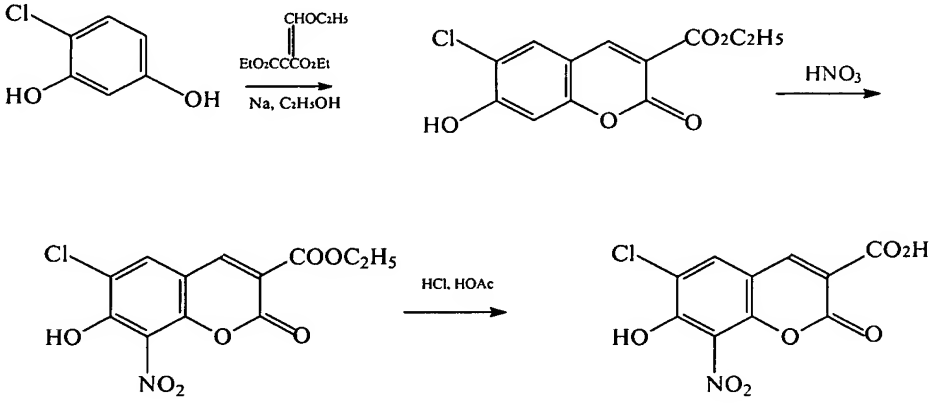
(13) 3-ethoxycarbonyl-5-methyl-6, 8-dinitro-7-hydroxycoumarin and 3-carboxy-5-methyl-6, 8-dinitro-7-hydroxy-coumarin



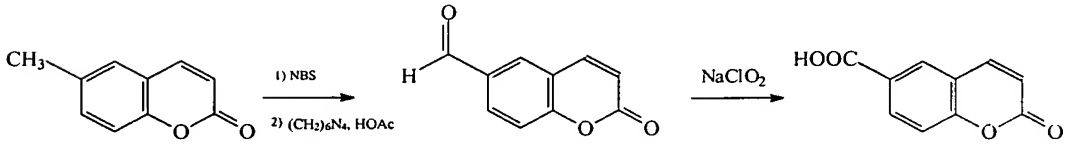
(14) 3-ethoxycarbonyl-6-ethyl-7-hydroxy-8-nitrocoumarin and 3-carboxy-6-ethyl-7-hydroxy-8-nitrocoumarin



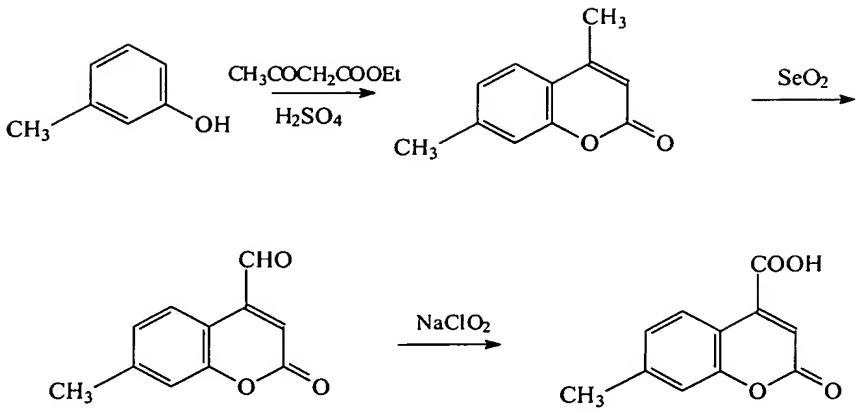
(15) 3-ethoxycarbonyl-6-chloro-7-hydroxy-8-nitrocoumarin and 3-carboxy-6-chloro-7-hydroxy-8-nitrocoumarin



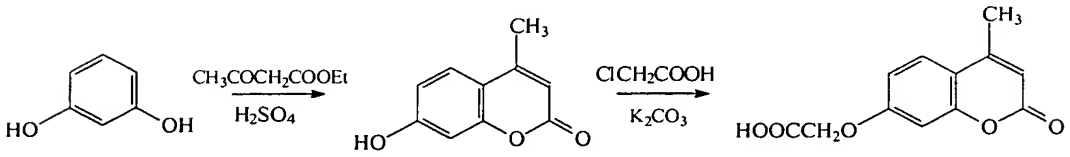
(16) 6-carboxycoumarin



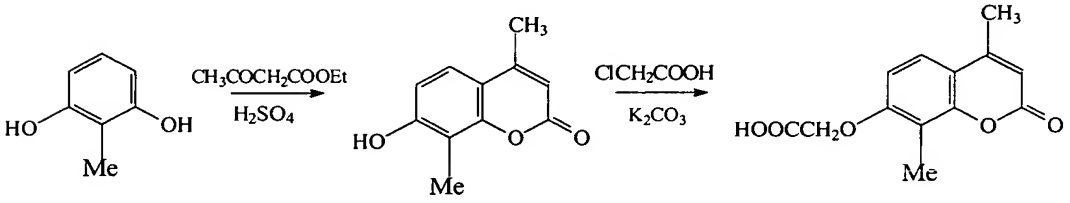
(17) 4-carboxy-7-methylcoumarin



(18) 4-methyl-7-carboxymethoxycoumarin



(19) 4, 8-dimethyl-7-carboxymethoxycoumarin



The following examples are intended to illustrate this invention, however these examples shall not be construed to limit the scope of the invention.

Example 1

Synthesis of 3-ethoxycarbonyl-6-chloro-7-hydroxy-8-nitro-coumarin (241)

2.75g (10.2mmol) of 3-ethoxycarbonyl-6-chloro-7-hydroxycoumarin was added into 10ml of

concentrated sulfuric acid, 1.74g (20.4mmol) of concentrated nitric acid was added in portions under the cooling of ice-salt bath, the reaction was monitored through thin-layer chromatography to confirm the completion, and ice was added to seize the reaction. The reaction mixture was then filtered and washed by water, dried to give 1.52g of the title compound (**241**).

^{1}H-NMR 300MHz (DMSO): 1.266 (t, 3H, CH_3), 4.232 (q, 2H, CH_2), 8.017 (s, 1H, 5-H), 8.593 (s, 1H, 4-H)

Compounds **229-246** in the tables were prepared following the same procedure.

Example 2

Synthesis of 3-ethoxycarbonyl-6-ethyl-7-hydroxy-8-nitro-coumarin (233)

Compound **233** was prepared following the preparation of compound **241,** except that 3-ethoxycarbonyl-6-ethyl-7-hydroxy-coumarin was nitrated to give the title compound **233**.

^{1}H-NMR 300MHz (DMSO): 1.262 (t, 3H, 6-ethyl-CH_3), 1.401 (t, 3H, ester -CH_3), 2.753 (q, 2H, 6-ethyl-CH_2), 3.988 (s, 3H, 7-OCH_3), 4.408 (q, 2H, ester-CH_2), 7.527 (s, 1H, 5-H), 8.479 (s, 1H, 4-H)

Example 3

Synthesis of 3-ethoxycarbonyl-6-nitro-7,8-dimethoxy-coumarin (227)

Compound **227** was prepared following the preparation of compound **241,** except that 3-ethoxycarbonyl-7,8-dimethoxycoumarin was nitrated to give the title compound **227**.

^{1}H-NMR 300MHz (DMSO): 1.397 (t, 3H, ester-CH_3), 4.063-4.118 (d, 6H, 7,8-OCH_3), 4.423 (q, 2H, ester-CH_2), 7.757 (s, 1H, 5-H), 9.252 (s, 1H, 4-H)

Example 4

Synthesis of 3-ethoxycarbonyl-6, 8-dinitro-7-methoxy-coumarin (239)

Compound was prepared following the preparation of compound **241,** except that 3-ethoxycarbonyl-7-methoxy-coumarin was bis-nitrated to give the title compound **239**.

^{1}H-NMR 300MHz (DMSO): 1.290 (t, 3H, ester-CH_3), 4.011 (s, 3H, 7-OCH_3), 4.292 (q, 2H, ester-CH_2), 8.873 (s, 1H, 4-H), 8.955 (s, 1H, 5-H)

Example 5

Synthesis of 3-ethoxycarbonyl-6, 8-dinitro-7-hydroxy-coumarin (237)

Compound **237** was prepared following the preparation of compound **241,** except that 3-ethoxycarbonyl-7-hydroxy-coumarin was bis-nitrated to give the title compound **237**.

^{1}H-NMR 300MHz (DMSO): 1.237 (t, 3H, ester-CH_3), 4.196 (q, 2H, ester-CH_2), 8.399 (s, 1H, 4-H), 8.636 (s, 1H, 5-H)

Example 6

Synthesis of 3-(3'-hydroxy-4'-carboxy-phenylamidocarbonyl)-6-ethyl-7-methoxy-coumarin (26)

248mg (1mmol) of 3-carboxy-6-ethyl-7-methoxycoumarin and 2ml of $SOCl_2$ was heated to complete the reaction. After that, $SOCl_2$ was

removed and 153mg (1mmol) of 4-aminosalicylic acid and 2 ml of pyridine was added. The mixture was heated to complete the reaction. The crude product was purified with DMSO to give 140mg of the title compound (**26**).

^{1}H-NMR 300MHz (DMSO): 1.142 (t, 3H, CH_3), 2.569 (q, 2H, CH_2), 3.906 (s, 3H, 7-OCH_3), 7.069 (d, 1H, 6'-H), 7.098 (s, 1H, 8-H), 7.509 (s, 1H, 2'-H), 7.758 (d, 1H, 5-H), 8.856 (s, 1H, 4-H), 10.848 (s, 1H, CONH), 11.399 (s, 1H, OH)

Compounds **1-109, 204-206, 208, 209, 213, 214, 217, 218, 220, 222-228** in table 1 and compounds **247-249** were prepared following the same procedure.

Example 7

Synthesis of

3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-6-ethyl-7-methoxy-coumarin (27)

Compound **27** was prepared following the preparation of compound **26**, except that 4-aminosalicylic acid was replaced by 5-amino-salicylic acid to give the title compound **27**.

^{1}H-NMR 500MHz (DMSO): 1.162 (t, 3H, ethyl-CH_3), 2.602 (q, 2H, ethyl-CH_2), 3.937 (s, 3H, 7-OCH_3), 6.786 (d, 1H, 5'-H), 7.178 (s, 1H, 6-H), 7.746 (d, 1H, 6'-H), 7.770 (s, 1H, 5-H), 8.239 (s, 1H, 2'-H), 8.834 (s, 1H, 4-H), 10.583 (s, 1H, CONH)

Element analysis for: $C_{20}H_{17}NO_7$

Calculated(%): C62.66, H4.47, N3.65

Found (%): C62.87, H4.49, N3.71

Example 8

Synthesis of

3-(m-carboxyphenylamidocarbonyl)-7-methoxycoumarin (2)

Compound **2** was prepared following the preparation of compound **26,** except that 3-carboxy-7-methoxy-coumarin reacted with m-aminobenzoic acid to give the title compound **2**.

Element analysis for: $C_{18}H_{13}NO_6 \cdot 1/2H_2O$

Calculated(%): C62.07, H4.05, N4.02

Found (%): C62.72, H3.74, N4.55

Example 9

Synthesis of

3-(3'-hydroxy-4'-carboxyphenylamidocarbonyl)-7-methoxy coumarin (7)

Compound **7** was prepared following the preparation of compound **26,** except that 3-carboxy-7-methoxy-coumarin reacted with 4-amino-salicylic acid to give the title compound **7**.

^{1}H-NMR 300MHz (DMSO): 3.91 (s, 3H, 7-OCH_3), 7.08 (d, 1H, 6-H), 7.11 (s, 1H, 6'-H), 7.53 (s, 1H, 2'-H), 7.77 (d, 1H, 5-H), 7.95 (d, 1H, 5'-H), 8.91 (s, 1H, 4-H), 10.83 (s, 1H, CONH), 11.40 (br, 1H, OH)

Element analysis for: $C_{18}H_{13}NO_7$

Calculated(%): C60.85, H3.69, N3.94

Found (%): C60.52, H3.59, N4.10

Example 10

Synthesis of

3-(3'-carboxy-4'-hydroxyphenylamidocarbonyl)-7-methoxy coumarin (8)

Compound **8** was prepared following the preparation of compound **26,** except that 3-carboxy-7-methoxy-coumarin reacted with 5-amino-salicylic acid to give the title compound **8**.

^{1}H-NMR 300MHz (DMSO): 3.906 (s, 3H, 7-OCH_3), 6.964 (d, 1H, 5'-H), 7.037 (d, 1H, 6-H), 7.083 (s, 1H, 8-H), 7.745 (d, 1H, 6'-H), 8.001 (d, 1H, 5-H), 8.234 (s, 1H, 2'-H), 8.877 (s, 1H, 4-H), 10.547 (s, 1H, CONH), 11.103 (br, OH)

Element analysis for: $C_{18}H_{13}NO_7$

Calculated(%): C60.85, H3.69, N3.94

Found (%): C60.50, H3.62, N3.64

Example 11

Synthesis of

3-[4'-(5"-methylisooxazol-3"-yl)-amidosulfonyl]phenylamido carbonyl]-7-methoxy-coumarin(19)

Compound **19** was prepared following the preparation of compound **26,** except that 3-carboxy-7-methoxy-coumarin reacted with sulfamethoxazole (SMZ) to give the title compound **19**.

Element analysis for: $C_{21}H_{17}N_3O_7S \cdot 1/2H_2O$

Calculated(%): C54.31, H3.91, N9.05

Found (%): C54.56, H3.49, N8.90

Example 12

Synthesis of

3-(3'-carboxypropylamidocarbonyl)-7-methoxycoumarin (203)

Compound **203** was prepared following the preparation of compound except that 3-carboxy-7-methoxy-coumarin reacted with γ-amino-butyric acid to give the title compound **203**.

^{1}H-NMR 300MHz (DMSO): 1.719 (t, 2H, 3'-CH_2), 2.235 (t, 2H, 2'-CH_2), 3.311 (t, 2H, 4'-CH_2), 3.861 (s, 3H, 7-OCH_3), 7.001 (d, 1H, 6-H), 7.074 (s, 1H, 8-H), 7.861 (d, 1H, 5-H), 8.771 (s, 1H, 4-H)

Element analysis for: $C_{15}H_{15}NO_6$

Calculated(%): C59.01, H4.95, N4.59

Found (%): C, 59.05 H4.60, N4.73

Example 13

Synthesis of

3-[4'-(5"-methylisooxazol-3")-amidosulfonyl]phenylamido carbonyl]-7-methoxy-8-methylcoumarin(55)

Compound **55** was prepared following the preparation of compound **26** except that 3-carboxy-7-methoxy-8-methylcoumarin reacted with SMZ to give the title compound **55**.

Element analysis for: $C_{22}H_{19}N_3O_7S$

Calculated(%): C56.28, H4.08, N8.95

Found (%): C56.61, H4.06, N9.01

Example 14

Synthesis of 3-(m-carboxymethylenoxy-phenylamidocarbonyl)-7,8-dimethoxycoumarin(64)

Compound **64** was prepared following the preparation of compound **26** except that 3-carboxy-7,8-dimethoxy-coumarin reacted with m-carboxy methylenoxyaniline to give the title compound **64**.

^{1}H-NMR 300MHz (DMSO): 3.852 (s, 3H, 8-OCH_3), 3.951 (s, 3H, 7-OCH_3), 4.641 (s, 2H, OCH_2), 6.676 (q, 1H, 5'-H), 7.198-7.420 (m, 3H, 4', 6', 6-H), 7.502 (s, 1H, 2'-H), 7.751 (d, 1H, 5-H), 8.853 (s, 1H, 4-H), 10.584 (s, 1H, CONH)

Element analysis for: $C_{20}H_{17}NO_8$

Calculated(%): C60.15, H4.29, N3.51
Found (%): C60.41, H4.65, N3.75

Example 15

Synthesis of 3-(4'-guanidinosulfonylphenylamidocarbonyl)-7,8-dimethoxy-coumarin (66)

Compound **66** was prepared following the preparation of compound **26** except that 3-carboxy-7,8-dimethoxy-coumarin reacted with sulfaguanidine (SG) to give the title compound **66.**

Element analysis for: $C_{19}H_{18}N_4O_7S \cdot 2H_2O$
Calculated(%): C47.30, H4.56, N11.61
Found (%): C47.34, H4.08, N11.00

Example 16

Synthesis of 3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-7,8-dimethoxy-coumarin (60)

Compound **60** was prepared following the preparation of compound **26** except that 3-carboxy-7,8-dimethoxy-coumarin reacted with 5-amino- salicylic acid to give the title compound **60**.

^{1}H-NMR 300MHz (DMSO): 3.849-3.947 (d, 6H, 7,8-bis-OCH_3), 6.962 (d, 1H, 5'-H), 7.233 (d, 1H, 6-H), 7.727-7.755 (d, 2H, 5, 6'-H), 8.210 (s, 1H, 2'-H), 8.813 (s, 1H, 4-H), 10.495 (s, 1H, CONH)
Element analysis for: $C_{19}H_{15}NO_8 \cdot 1/4H_2O$
Calculated(%): C58.61, H4.01, N3.59
Found (%): C58.27, H3.86, N3.92

Example 17

Synthesis of
3-(benzoylhydrazinocarbonyl)-5-methyl-7-methoxycoumarin (210)

Compound **210** was prepared following the preparation of compound **26** except that 3-carboxy-5-methyl-7-methoxy-coumarin reacted with benzoyl hydrazine to give the title compound **210**.

^{1}H-NMR 300MHz (DMSO): 2.482 (s, 3H, 5-CH_3), 3.888 (s, 3H, 7-OCH_3), 6.979 (d, 2H, 6, 8-H), 7.477-7.583 (q, 2H, 3', 5'-H), 7.500 (t, 1H, 5'-H), 7.889 (d, 2H, 2', 6'-H), 8.792 (s, 1H, 4-H), 10.24 (s, 1H, CONH), 10.868 (s, 1H, CONH)

Example 18

Synthesis of 3-(isonicotinoylhydrazinocarbonyl)-5-methyl-7-methoxy coumarin (213)

Compound **213** was prepared following the preparation of compound **26** except that 3-carboxy-5-methyl-7-methoxy-coumarin reacted with isoniazid to give the title compound **213**.

^{1}H-NMR 300MHz (DMSO): 2.553 (s, 3H, 5-CH_3), 3.878 (s, 3H, 7-OCH_3), 6.979 (d, 2H, 6, 8-H), 7.935 (d, 2H, 3', 5'-H), 8.781 (s, 1H, 4-H), 10.545 (s, 1H, CONH), 11.362 (s, 1H, CONH)

Example 19

Synthesis of 3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-5-methyl-7-methoxycoumarin (74)

Compound **74** was prepared following the preparation of compound **26** except that 3-carboxy-5methyl-7-methoxy-coumarin reacted with 5-amino-salicylic acid to give the title compound **74**.

Element analysis for: $C_{19}H_{15}NO_7$

Calculated(%): C61.79, H4.09, N3.79

Found (%): C61.57, H4.07, N3.81

Example 20

Synthesis of

3-(3'-hydroxy-4'-carboxy-phenylamidocarbonyl)-6-chloro-7-methoxy-coumarin (87)

Compound **87** was prepared following the preparation of compound **26** except that 3-carboxy-6-chloro-7-methoxy-coumarin reacted with 4-amino- salicylic acid to give the title compound **87**.

^{1}H-NMR 300MHz (DMSO): 3.996 (s, 3H, 7-OCH_3), 7.114 (d, 1H, 6'-H), 7.376 (s, 1H, 8-H), 7.485 (s, 1H, 2'-H), 7.768 (d, 1H, 5'-H), 8.146 (s, 1H, 5-H), 8.839 (s, 1H, 4-H), 10.721 (s, 1H, CONH)

Element analysis for: $C_{18}H_{12}ClNO_7$

Calculated(%): C55.47, H3.11, N3.59

Found (%): C55.97, H3.13, N4.48

Example 21

Synthesis of

3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-6-chloro-7-methoxy-coumarin (88)

Compound **88** was prepared following the preparation of compound **26** except that 3-carboxy-6-chloro-7-methoxy-coumarin reacted with 5-amino- salicylic acid to give the title compound **88.**

^{1}H-NMR 300MHz (DMSO): 4.010 (s, 3H, 7-OCH_3), 6.968 (d, 1H, 5'-H), 7.380 (s, 1H, 8-H), 7.752 (d, 1H, 6'-H), 8.153 (s, 1H, 5-H), 8.211 (s, 1H, 2'-H), 8.817 (s, 1H, 4-H), 10.475 (s, 1H, CONH)

Element analysis for: $C_{18}H_{12}ClNO_7$

Calculated(%): C55.47, H3.11, N3.59

Found (%): C55.60, H3.18, N4.1

Example 22

Synthesis of

3-(3'-hydroxy-4'-carboxy-phenylamidocarbonyl)-6-bromo-7-methoxy-coumarin (96)

Compound **96** was prepared following the preparation of compound **26** except that 3-carboxy-6-bromo-7-methoxy-coumarin reacted with 4-amino- salicylic acid to give the title compound **96**.

^{1}H-NMR 300MHz (DMSO): 3.996 (s, 3H, 7-OCH_3), 7.118 (d, 1H, 6'-H), 7.343 (s, 1H, 8-H), 7.496 (s, 1H, 2'-H), 7.774 (d, 1H, 5'-H), 8.306 (s, 1H, 5-H), 8.846 (s, 1H, 4-H), 10.722 (s, 1H, CONH)

Example 23

Synthesis of 3-(4'-guanidinosulfonylphenylamidocarbonyl)-6-ethyl-7-methoxy-coumarin (32)

Compound **32** was prepared following the preparation of compound **26** except that 3-carboxy-6-ethyl-7-methoxy-coumarin reacted with SG to give the title compound **32**.

^{1}H-NMR 300MHz (DMSO): 1.148 (t, 3H, ethyl-CH_3), 2.572 (q, 2H, ethy-CH_2), 3.896 (s, 3H, OCH_3), 6.690 (br, 4H, guanidino-H), 7.125 (s, 1H, 8-H), 7.709 (s, 1H, 5-H), 7.739 (q, 4H, Ar-H), 8.827 (s, 1H, 4-H), 10.841 (s, 1H, CONH)

Element analysis for: $C_{20}H_{20}N_4O_6S \cdot 1/4H_2O$

Calculated(%): C53.55, H4.60, N12.48

Found (%): C53.49, H4.63, N12.40

Example 24

Synthesis of 3-(4'-guanidinosulfonylphenylamidocarbonyl)-6-chloro-7-methoxy-coumarin (92)

Compound **92** was prepared following the preparation of compound **26** except that 3-carboxy-6-chloro-7-methoxy-coumarin reacted with SG to give the title compound **92**.

^{1}H-NMR 300MHz (DMSO): 3.999 (s, 3H, 7-OCH_3), 7.407 (s, 1H, 8-H), 7.776 (q, 4H, Ar-H), 8.172 (s, 1H, 5-H), 8.860 (s, 1H, 4-H), 10.787 (s, 1H, CONH)

Element analysis for: $C_{18}H_{15}ClN_4O_6S$

Calculated(%): C47.95, H3.35, N12.43

Found (%): C47.54, H3.45, N12.15

Example 25

Synthesis of

3-(3'-hydroxy-4'-carboxy-phenylamidocarbonyl)-7-methoxy-8-methyl-coumarin (43)

Compound **43** was prepared following the preparation of compound **26** except that 3-carboxy-7-methoxy-8-methyl-coumarin reacted with 4-amino-salicylic acid to give the title compound **43**.

^{1}H-NMR 300MHz (DMSO): 2.215 (s, 3H, 8-CH_3), 3.912 (s, 3H, 7-OCH_3), 7.081(d, 1H, 6'-H), 7.182(d, 1H, 6-H), 7.612(s, 1H, 2'-H), 7.747(d, 1H, 5-H), 7.872(d, 1H, 5'-H), 8.834(s, 1H, 4-H), 10.813(s, 1H, CONH)

Element analysis for: $C_{19}H_{15}NO_7 \cdot 1/2H_2O$

Calculated(%): C60.32, H4.26, N3.70

Found (%): C60.26, H4.03, N4.14

Example 26

Synthesis

of3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-7-methoxy-8-methyl-coumarin (44)

Compound **44** was prepared following the preparation of compound **26** except that 3-carboxy-7-methoxy-8-methylcoumarin reacted with 5-amino-salicylic acid to give the title compound **44**

^{1}H-NMR 300MHz (DMSO): 2.209(s, 3H, 8-CH_3), 3.753(s, 3H, 7-OCH_3), 6.959(d, 1H, 5'-H), 7.168(d, 1H, 6-H), 7.723(d, 1H, 6'-H), 7.848(d, 1H, 5-H), 8.197(s, 1H, 2'-H), 8.794(s, 1H, 4-H), 10.504(s, 1H, CONH)

Element analysis for: $C_{19}H_{15}NO_7 \cdot 1/2H_2O$

Calculated(%): C60.32, H4.26, N3.70

Found (%): C59.66, H3.92, N3.81

Example 27

Synthesis of

3-(4'-methoxy-phenylamidocarbonyl)-6-nitro-7-hydroxy-8-methyl-coumarin (146)

160mg (0.604mmol) of 3-carboxy-6-nitro-7-methoxy-8-methyl-coumarin and 2ml of thionyl chloride was heated to complete the reaction. Extra thionyl chloride was removed and 74.3mg (0.604mmol) of p-anisidine, 1 ml of pyridine and 1ml of DMF were added therein and the so-obtained mixture was heated to complete the reaction. The reaction mixture was then filtered and washed by water, diluted hydro chloride, water and ethanol, respectively, dried and purified with glacial acetic acid to give 170mg of the title compound (**146**).

^{1}H-NMR 300MHz (DMSO): 2.280(s, 3H, Ar-CH_3), 3.740(s, 3H, OCH_3), 6.941(d, 2H, 3', 5'-H), 7.621(d, 2H, 2', 6'-H), 8.673(s, 1H, 5-H), 8.897(s, 1H, 4-H), 10.374(s, 1H, CONH)

Compounds **110-203, 225-228** were prepared following the same procedure.

Example 28

Synthesis of 3-(4'-guanidinosulfonylphenylamidocarbonyl)-6-nitro-7-methoxy-8-methyl-coumarin (169)

Compound **169** was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7-methoxy-8-methylcoumarin reacted with SG, and purified with DMF to give the title compound **169**.

^{1}H-NMR 300MHz (DMSO): 2.382(s, 3H, 8-CH_3), 3.940(s, 3H, 7-OCH_3), 6.677(br, 4H, guanidino-H), 7.790(q, 4H, Ar-H), 8.593(s, 1H, 5-H), 8.903(s, 1H, 4-H), 10.707(s, 1H, CONH)

Element analysis for: $C_{19}H_{17}N_5O_8S \cdot 1/2H_2O$

Calculated(%): C47.10, H3.75, N14.46

Found (%): C47.27, H3.73, N14.58

Example 29

Synthesis of 3-(4'-carboxy-phenylamidocarbonyl)-6-nitro-7,8-dimethoxy coumarin (110)

Compound **110** was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7,8-methoxycoumarin reacted with p-amino-benzoic acid to give the title compound **110**.

^{1}H-NMR 300MHz (DMSO): 3.99-4.06(q, 6H, 7,8-bis-OCH_3), 7.82(d, 2H, J=8.7, Ar-H), 7.9(d, 2H, J=8.7, Ar-H), 8.15(s, 1H, 5-H), 9.09(s, 4-H)10.91(s, 1H, CONH)

Example 30

Synthesis of 3-(3'-carboxy-phenylamidocarbonyl)-6-nitro-7,8-dimethoxy-coumarin (111)

Compound **111** was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7,8-dimethoxycoumarin reacted with m-amino-benzoic acid to give the title compound **111**.

^{1}H-NMR 300MHz (DMSO): 3.97-4.05(q, 6H, 7,8-bis-OCH_3), 7.49(t, 1H, 5'-H), 7.67(d, 1H, 6'-H), 7.76(d, 1H, 4'H), 7.93(s, 1H, 2'-H), 8.32(s, 1H, 5-H), 9.08(s, 1H, 4-H), 10.66(s, 1H, CONH)

Example 31

Synthesis of 3-[4'-(5", 6"-dimethoxypyrimidine-4")-amidosulfonyl phenylamidocarbonyl]-6-nitro-7,8-dimethoxycoumarin (123)

Compound **123** was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7,8-methoxycoumarin reacted with sulfadoxine (SDM) to give the title compound **123**.

^{1}H-NMR 300MHz (DMSO): 3.694(s, 3H, pyrimidine-OCH_3), 3.894(s, 3H, 8-OCH_3), 4.064(s, 3H, 7-OCH_3), 7.886-7.996(q, 4H, Ar-H), 7.974(s, 1H, 2"-H), 8.109(s, 1H, 5-H), 9.092(s, 1H, 4-H), 10.791(s, 1H, CONH), 10.947(br, 1H, SO_2NH)

Example 32

Synthesis of

3-(3'-hydroxy-4'-carboxyphenylamidocarbonyl)-6-nitro-7-hydroxy-8-methyl-coumarin (148)

Compound **148** was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7-hydroxy-8-methylcoumarin reacted with 4-aminosalicylic acid to give the title compound **148**.

^{1}H-NMR 300MHz (DMSO): 2.27(s, 3H, Ar-CH_3), 7.11(dd, 1H, J=7.8Hz, 1.8Hz, 6'-H), 7.498(d, 1H, J=1.8Hz, 2'-H), 7.775(d, 1H, J=7.8, 5'-H), 8.65(s, 1H, 5-H), 8.892(s, 1H, 4-H), 10.69(s, 1H, CONH)

Example 33

Synthesis of

3-(3'-carboxy-4'-hydroxy-phenylamidocarbonyl)-6-nitro-7-hydroxy-8-methyl-coumarin (149)

Compound **149**was prepared following the preparation of compound **146** except that 3-carboxy-6-nitro-7-hydroxy-8-methylcoumarin reacted with 5-aminosalicylic acid to give the title compound **149**.

^{1}H-NMR 300MHz (DMSO): 2.268(s, 3H, Ar-H), 6.971(d, 1H, J=8.7Hz, 5'-H), 7.747(dd, 1H, J=8.7Hz, 2.7Hz, 6'-H), 8.208(d, 1H, J=2.7Hz, 2'-H), 8.658 (s, 1H, 5-H), 8.867(s, 1H, 4-H), 10.403(s, 1H, CONH)

Element analysis for: $C_{18}H_{12}N_2O_9 \cdot 1/2H_2O$

Calculated(%): C 52.83 , H 3.22, N 6.85

Found (%): 52.92 , 3.26, 6.99

Example 34

Synthesis of

3-[4'-(2"-pyrimidinylamidosulfonyl)phenylamidocarbonyl]-5-methyl-6,8-dinitro-7-hydroy-coumarin (200)

Compound **200** was prepared following the preparation of compound **146** except 3-carboxy-5-methyl-6,8-nitro-7-hydroxycoumarin reacted with sulfadiazine (SD) to give the title compound **200**.

^{1}H-NMR 300MHz (DMSO): 2.291(s, 3H, 5-CH_3), 7.025(t, 1H, 5"-H), 7.884(q, 4H, Ar-H), 8.483(d, 2H, 4", 6"-H), 8.640(s, 1H, 4-H), 10.705(s, 1H, CONH)

Example 35

Synthesis of 3-(4'-amidosulfonylphenylamidocarbonyl)-5-methyl-6, 8-dinitro-7-hydroxy-coumarin (198)

Compound **198** was prepared following the preparation of compound **146** except that 3-carboxy-5-methyl-6, 8-nitro-7-hydroxycoumarin reacted with sulfanilamide to give the title compound **198**.

^{1}H-NMR 300MHz (DMSO): 2.254(s, 3H, 5-CH_3), 7.240(br, 2H, NH_2), 7.788(q, 4H, Ar-H), 8.666(s, 1H, 4-H), 10.676(s, 1H, CONH)

Example 36

Synthesis of 3-(2'-thiazolamidosulfonylphenylamidocarbonyl)-5-methyl-6, 8-dinitro-7-hydroxy-coumarin (201)

Compound **201** was prepared following the preparation of compound **146** except that 3-carboxy-5-methyl-6, 8-nitro-7-hydroxycoumarin reacted with sulfathiazole (ST) to give the title compound **201**.

^{1}H-NMR 300MHz (DMSO): 2.291(s, 3H, 5-CH_3), 6.802(d, 1H, thiazole-H), 7.225(d, 1H, thiazolyl-H), 7.737(q, 4H, Ar-H), 8.651(s, 1H, 4-H), 10.667(s, 1H, CONH)

Example 37

Synthesis of 3-(4'-guanidinosulfonylphenylamidocarbonyl)-5-methyl-6, 8-dinitro-7-hydroxy-coumarin (199)

Compound **199** was prepared following the preparation of compound **146** except that

3-carboxy-5-methyl-6,8-dinitro-7-hydroxycoumarin reacted with SG to give the title compound **199**.

^{1}H-NMR 300MHz (DMSO): 2.293(s, 3H, 5-CH_3), 6.685(br, 4H, guanidino-H), 7.746(q, 4H, Ar-H), 8.657(s, 1H, 4-H), 10.647(s, 1H, CONH)

Example 38

Synthesis of

3-(2'-phenyl-1',3',4'-oxadiazol-5'-yl)-7-methoxy-8-methyl coumarin (216)

295mg (0.84mmol) of 3-(benzoylhydrazinocarbonyl)-7-methoxy-8-methylcoumarin reacted with 4.6ml phosphorus oxychloride at 100°C for 5 hours, and the reaction mixture was left to be cool and then poured into ice-water, filtrated, washed with water, and dried. 290mg of the crude product was obtained, and then the crude product was purified with DMF to give 160mg of the title compound **216**.

^{1}H-NMR 300MHz (DMSO): 2.252(s, 3H, 8-CH_3), 3.968(s, 3H, 7-OCH_3), 7.174(d, 1H, 6-H), 7.634(m, 3H, Ar'-H), 7.812(d, 1H, 5-H), 8.088(m, 2H, Ar'-H), 8.874(s, 1H, 4-H)

Compounds **206, 207, 210-212, 215, 216, 219** and **221** in table 2 were prepared following the same procedure.

Example 39

Synthesis of

3-(2'-phenyl-1',3',4'-oxadiazol-5'yl)-7-methoxycoumarin (206)

Compound **206** was prepared following the preparation of compound **216** except that

3-(benzoylhydrazinocarbonyl)-7-methoxycoumarin reacted with phosphorus oxychloride to give the title compound **206**.

^{1}H-NMR 300MHz (DMSO): 3.929(s, 3H, 7-OCH_3), 7.021(d, 1H, 6-H), 7.085(s, 1H, 8-H), 7.599-7.668(m, 3H, Ar-H), 7.871(d, 1H, 5-H), 8.095(m, 2H, Ar-H), 8.898(s, 1H, 4-H)

Element analysis for: $C_{18}H_{12}N_2O_4$

Calculated(%): C67.49, H3.78, N8.75

Found (%): C67.57, H3.98, N8.41

Example 40

Synthesis of 3-[(2'-pyridyl-4")-1',3',4'-oxadiazol-5'yl]-6-hexyl-7-methoxy coumarin (221)

Compound **221** was prepared following the preparation of compound **216** except that 3-(isonicotinoylhydrazinocarbonyl)-6-hexyl-7-methoxy coumarin reacted with phosphorus oxychloride to give the title compound **221**.

^{1}H-NMR 300MHz (DMSO): 0.869(t, 3H, hezyl -CH_3), 1.240(br, 6H, hezyl-CH_2), 1.574(t, 2H, hexyl-CH_2), 2.734(t, 2H, hezyl-CH_2), 3.959(s, 3H, 7-OCH_3), 7.116(s, 1H, 8-H), 7.699(s, 1H, 5-H), 8.070(br, 2H, pyridyl -H), 8.920(br, 2H, pyridyl-H), 8.921(s, 1H, 4-H)

Element analysis for: $C_{23}H_{23}N_3O_4 \cdot 3H_2O$

Calculated(%): C60.12, H6.36, N9.15

Found (%): C59.51, H5.51, N8.96

Example 41

Synthesis of 4-methyl-7-(4'-ethoxycarbonylphenylamidocarbonyl-methylenoxy)coumarin (255)

60mg (0.256mmol) of 4-methyl-7-carboxy-methylenoxycoumarin and 2ml of thionyl chloride

was heated to complete the reaction. Extra thionyl chloride was removed and the residue was dissolved in 5ml of methylene chloride. 44 mg (0.267mmol) of ethyl 4-amino-benzoate in 5ml methylene chloride and 3ml of pyridine were added therein and the reaction mixture was stirred for 0.5 hours to precipitate the solid and the stirration was continued for additional 1 hour. The product was filtrated, washed with methylene chloride, and dried to give 80mg of the title compound (**255**).

^{1}H-NMR 300MHz (DMSO): 1.293(t, 3H, ester-methyl); 2.389(s, 3H, 4-methyl);4.269(q, 2H, ester-CH_2), 4.881(s, 2H, OCH_2), 6.219(s, 1H, 3-H), 7.018(d, 1H, 8-H), 7.056(d, 1H, 6-H), 7.712(d, 1H, 5-H), 7.760(d, 2H, 2', 6'-H), 7.919(d, 2H, 3', 5'-H), 10.479(s, 1H, CONH)

Element analysis for: $C_{21}H_{19}NO_6$

Calculated(%): C66.13, H5.02, N3.67

Found (%): C66.26, H4.91, N3.81

Compounds **250-264** in table 2 were prepared following the same procedure.

Example 42

Synthesis of

4-methyl-7-phenylamidocarbonyl-methylenoxycoumarin (248)

Compound **248** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by aniline to give the title compound **248**.

^{1}H-NMR 300MHz (DMSO): 2.377(s, 3H, 4-CH_3), 4.825(s, 2H, 7OCH_2), 6.208(s, 1H, 3-H), 6.997(m, 3H, 4', 6, 8-H), 7.306(t, 2H, 3', 5'-H), 7.593(d, 2H, 2', 6'-H), 7.711(d, 1H, 5-H), 10, 144(s, CONH)

Element analysis for: $C_{18}H_{15}NO_4$

Calculated(%): C69.89, H4.89, N4.53

Found (%): C69.61, H4.891, N4.58

Example 43

Synthesis of

4-methyl-7-(4'-carboxyphenylamidocarbonyl-methylenoxy) coumarin (252)

Compound **252** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by p-amino-benzoic acid to give the title compound **252**.

^{1}H-NMR 300MHz (DMSO): 2.404(s, 3H, 4-CH_3), 4.899(s, 2H, 7-OCH_2), 6.235(s, 1H, 3-H), 7.036(s, 1H8-H), 7.073(d, 1H, 6-H), 7.713(d, 1H, 5-H), 7.739-7.924(q, 4H, Ar-H), 10.491(s, 1H, CONH)

Element analysis for: $C_{19}H_{15}NO_6 \cdot 1/4H_2O$

Calculated(%): C63.77, H4.37, N3.92

Found (%): C63.76, H4.28, N4.24

Example 44

Synthesis of

4-methyl-7-(4'-hydroxyphenylamidocarbonyl-methylenoxy) coumarin (249)

Compound **249** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by p-amino-phenol to give the title compound **249**.

^{1}H-NMR 300MHz (DMSO): 2.084(s, 3H, 4-CH_3), 4.781(s, 2H, 7-OCH_2), 6.230(s, 1H, 3-H), 6.705-7.390(q, 4H, Ar-H), 7.014(s, 1H, 8-H), 7.060(d, 1H, 6-H), 7.723(d, 1H, 5-H), 9.905(s, 1H, CONH)

Element analysis for: $C_{18}H_{15}NO_5$

Calculated(%): C66.45, H4.65, N4.31

Found (%): C66.14, H4.62, N4.32

Example 45

Synthesis of

4-methyl-7-(3'-carboxy-4'-hydroxyphenylamidocarbonyl-methylenoxy)coumarin (261)

Compound **261** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by 5-amino-salicylic acid to give the title compound **261**.

^{1}H-NMR 300MHz (DMSO): 2.495(s, 3H, 4-CH_3), 4.818(s, 2H, 7-OCH_2), 6.233(s, 1H, 3-H), 6.940(d, 1H, 6-H), 7.052(s, 1H, 8-H), 7.077(d, 1H, 5'-H),

Element analysis for: $C_{19}H_{15}NO_7$

Calculated(%): C61.79, H4.09, N3.79

Found (%): C61.49, H3.96, N3.86

Example 46

Synthesis of 4-methyl-7-(3'-trifluoromethylphenylamidocarbonyl-methylenoxy)coumarin (257)

Compound **257** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by 3-fluoromethyl aniline to give the title compound **257**.

^{1}H-NMR 300MHz (DMSO): 2.389(s, 3H, 4-CH_3), 4.872(s, 2H, 7-OCH_2), 6.220(s, 1H, 3-H), 7.027-7.075(m, 2H, 6, 8-H), 7.429(d, 1H, 6'-H), 7.567(t, 1H, 5'-H), 7.719(d, 1H, 5-H), 7.857(d, 1H, 4'-H), 8.096(s, 1H, 2'-H), 10.446(s, 1H, CONH)

Element analysis for: $C_{19}H_{14}F_3NO_4$

Calculated(%): C60.48, H3.74, N3.71

Found (%): C60.17, H3.45, N3.79

Example 47

Synthesis of 4-methyl-7-(3'-trifluoromethyl-4'-nitrophenylamido carbonylmethylenoxy)coumarin (258)

Compound **258** was prepared following the preparation of compound **255** except that ethyl 4-amino-benzoate was replaced by 3-fluoromethyl- 4'-nitro-aniline to give the title compound **258**.

^{1}H-NMR 300MHz (DMSO): 2.409(s, 3H, 4-CH_3), 4.955(s, 2H, 7-OCH_2), 6.243(s, 1H, 3-H), 7.061(s, 1H, 8-H), 7.086(d, 1H, 6-H), 7.734(d, 1H, 5'-H), 8.127(d, 1H, 6'-H), 8.215(d, 1H, 5-H), 8.331(s, 1H, 2'-H), 10.945(s, 1H, CONH)

Element analysis for: $C_{19}H_{13}F_3N_2O_6 \cdot 1/2H_2O$

Calculated(%): C52.91, H3.27, N6.50

Found (%): C53.19, H3.05, N6.76

Example 48

Synthesis of 4, 8-dimethyl-7-(3'-trifluoromethylphenylamidocarbonyl-methylenoxy)coumarin (262)

Compound **262** was prepared following the preparation of compound **255** except that 4,8-dimethyl-7-carboxy-methylenoxycoumarin reacted with 3-fluoromethylaniline to give the title compound **262**.

H-NMR 300MHz (DMSO): 2.291(s, 3H, 8-CH_3), 2.392(s, 3H, 4-CH_3), 4.934(s, 2H, 7-OCH_2), 6.237(s, 1H, 3-H), 7.002(d, 1H, 6-H), 7.440(d, 1H, 6'-H), 7.564(d, 1H, 5'-H), 7.603(d, 1H, 5-H), 7.816(d, 1H, 4'-H), 8.103(s, 1H, 2'-H), 10.503(s, 1H, CONH)

Element analysis for: $C_{20}H_{16}F_3NO_4$

Calculated(%): C61.38, H4.12, N3.58

Found (%): C61.16, H4.03, N3.67

Example 49

Synthesis of

4,8-dimethyl-7-(3'-hydroxy-4-carboxyphenylamidocarbonyl methylenoxy)-coumarin (264)

Compound **264** was prepared following the preparation of compound **255** except that 4,8-dimethyl-7-carboxy-methylenoxycoumarin reacted with 4-amino-salicylic acid to give the title compound **264**.

^{1}H-NMR 300MHz (DMSO): 2.270(s, 3H, 8-CH_3), 2.371(s, 3H, 4-CH_3), 4.931(s, 2H, 7-OCH_2), 6.215(s, 1H, 3-H), 6.958(d, 1H, 6-H), 7.087(d, 1H, 6'-H), 7.337(s, 1H, 2'-H), 7.546(d, 1H, 5'-H), 7.717(d, 1H, 5-H), 10.455(s, 1H, CONH)

Element analysis for: $C_{20}H_{17}NO_7$

Calculated(%): C62.66, H4.47, N3.65

Found (%): C62.43, H4.43, N3.88

Example 50

Synthesis of 6-(4'-ethyloxycarbophenylamidocarbonyl)coumarin (265)

A mixture of 95mg (0.5mmol) of 6-carboxycoumarin and phosphorous pentachloride in 50ml toluene was refluxed for 1 hour and the reaction mixture was concentrated. To the residue obtained, 83mg (0.5mmol) of ethyl p-amino benzoate and 1 ml of pyridine were added and the reflux was continued for additional 10 minutes. The reaction mixture was cooled down and acidified with hydrochloric acid to obtain a solid, which was purified with ethanol to give 100mg of the title compound **265**

^{1}H-NMR 300MHz (DMSO): 1.31(t, 3H, ester-CH_3), 4.28(q, 2H, ester-CH_2), 6.59(d, 1H, 3-H), 7.55(d, 1H, 8-H), 7.92(d, 2H, Ar'-H), 7.96(d, 2H, Ar'-H), 8.16(m, 2H, 4, 7-H), 8.34(d, 1H, 5-H), 10.68(s, 1H, CONH)

Element analysis for: $C_{19}H_{15}NO_5 \cdot 1/2H_2O$

Calculated(%): C65.80, H4.65, N4.04

Found (%): C66.07, H4.59, N4.06

Compound **266** was prepared following the same procedure.

Pharmacologic Experiments

Example 1. TGF-β-induced cell growth inhibition of the test compounds on Mink pulmonary epithelial cells

Mink pulmonary epithelial cells were seeded in 24 well-plate at a density of 3×10^4 cells/well and cultured in modified Eagle's medium (MEM) containing 10% fetal bovine serum in 37°C and 5%CO_2. Next day the serum was replaced by a MEM containing 0.2% fetal bovine serum. After 24 hours, the medium was replaced with fresh medium containing 10 pmol/L TGF-β1 and test compounds, and incubated for 24h. [^{3}H]Thymidine was added to the medium 2 hours before the termination of incubation. After removing the medium, cells were washed with PBS, dissolved in 0.5mol/L NaOH, and radioactivity was measured. The inhibitory effects of test compounds are represented as the percentage of Thymidine uptake recovery (Table 3).

Tab. 3 TGF-β-induced cell growth inhibition of tested compounds in Mink Lung epithelial cells

No. of tested compounds(10μg/ml)	26	92	73	7	2
Inhibition recovery rate on cell growth(%)	70.7	95.0	15.1	67.1	27.1

Example 2. TGF-β receptor binding antagonism assay of test compounds

Balb/c 3T3 cells were seeded in 24 well-plate and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, in 37°C and 5%CO_2, for 2-4 days. When cells were at a near-confluent, medium was changed to the binding buffer (50 mmol/L HEPES containing NaCl, KCl, $MgSO_4$ and $CaCl_2$). The assay was initiated by addition of 50 pmol/L [^{125}I]TGF-β1 and test compounds. After incubation for 210-240 minutes, the medium was removed, and cells were washed with ice-cold binding buffer. Non-specific bindings in the presence of 10 nmol/L TGF-β1 were determined. The cells were then solubilized using buffer containing Triton X-100 and the radioactivity was measured (see Table 4).

Tab. 4 TGF-β receptor binding antagonism of tested compounds in Balb/c 3T3 cells

Compounds (10μg/ml)	Inhibition Rate (%)	IC_{50} (μg/ml)	Compounds (10μg/ml)	Inhibition Rate (%)	IC_{50} μg/ml)
1	32.2		42	4.1	
2	74.1	13.8	49	15.5	
3	11.7		55	52.3	
6	-6.0		66	52.3	
7	94.2	7.8	67	16.2	
9	11.4		73	60.0	
12	5.1		79	16.2	

14	35.9		83	21.2	
21	10.1		87	91.1	
22	37.4		88	111.2	5.3
25	11.6		91	34.7	
26	95.4	8.5	92	106.4	
27	77.2		93	29.7	
31	29.2		96	82.3	
33	32.1		104	42.8	
34	36.4		206	-0.7	
37	41.4				

Example 3. Protection of tested compound against chronic renal failure caused by 5/6 nephrectomy in rats

The model on chronic renal failure induced by partial renal ablation in rats was set up according to the *Guidelines of Pre-clinic Research for New Drugs*.

Male Wistar rats with initial body weights of ~200g, anesthetized with 35mg/kg of pentobarbital i.p., the right kidney was removed, and the upper and lower pole parenchymas of the left kidney were resected, stop bleeding, closed the abdominal and sutured. Four weeks following surgery, the BUN, creatinine and urinary protein in rats were determined. Angiotensin II (AII) level was determined using radioimmunoassay and TGF-β1 using ELISA methods. Rats were randomly divided into seven groups, 30/group, with sham, model, Benazepril (4mg/kg/day) and Losartan (10mg/kg/day) as positive control, and compound 149 groups (7.5mg/kg/day, 15mg/kg/day and 30mg/kg/day, respectively). All the groups were administered intragastrically, once per day and 6 (six) times per week, till 16 weeks post surgery.

Body weights were weighed to observe the growth of the rats. The above indexes were determined every 4 weeks up to the 16^{th} week following the initiation of drug administration, and at each time, a number of animals were sacrificed and the kidneys were harvested for pathological analysis.

Pathological lesions in chronic renal failure models are mainly glomerulosclerosis and interstitial fibrosis. According to the damage extents, glomerulosclerosis is divided into five grades (0~IV). 0 grade means no glomerulus pathological changes at all, and IV grade means ultimately glomerular sclerosis and glassy pathological changes. 50 glomerulus were observed in each animal kidney tissue slide, and percentage of various grade in each group was calculated based on the above five grades.

4 weeks after nephrectomy, BUN in serum increased by 111.12% ($P<0.01$), urinary protein concentration increased by 86.13% ($P<0.01$), and TGF-β1 level increased by 70.84% ($P<0.02$).

12 weeks after nephrectomy (8 weeks since administration), morphological results demonstrated that the rates of 0 grade glomerylus in residual kidney in 30mg/kg compound 149($P<0.05$) and Losartan ($P<0.05$) groups were higher than that of the model group with significant difference. Glomerulus pathological scores were less than that of the model group significantly. The inflammatory cell infiltration existed in some animal kidney tissues of the Benazepril group was severe, and nephrotubular enlargement as well as protein-like substance also appeared.

16 weeks after nephrectomy (12 weeks since administration), pathological results indicated that the number of grade III glomerulus in the 30mg/kg group of compound 149 and the Losartan group was less than that of the model group significantly ($P<0.01$ and $P<0.05$,

respectively). The glomerulus pathological score in the positive Benazepril group was the highest, and the inflammatory cell infiltration in kidney matrix was medium-severe, interstitial fibrosis, nephrotubular enlargement as well as protein-like substance existed.

The results are shown in Tables 5A-E.

Tab.5 Protection of test compound on chronic renal failure induced by 5/6 nephrectomy in rats

A. The change of serum creatinine and BUN concentration in 8th week after administration (following 12 weeks after nephrectomy)

Groups	Dose (mg/kg)	Scr. (mg/dL)	Change Rate (%)	BUN (mg/dL)	Change Rate(%)
Sham	-	2.08±0.742		13.00±2.326	125.90↑
Model	-	3.06±0.768	47.93↑	29.37±3.079#	28.23↑
Benazepril	4	3.54±1.140	15.36↑	37.66±8.895	12.70↓
Losartan	10	2.34±0.268*	23.46↓	25.64±5.116	23.89↓
Compound	7.5	2.14±0.500*	30.26↓	22.35±3.120*	11.33↓
149	15	1.80±0.550*	41.34↓	26.04±4.234	3.98↑
	30	1.89±0.184	38.20↓	30.54±11.697	

Note: *P<0.05, compared with the model group; #P<0.05, compared with the sham group; ↑: increase; ↓: decrease.

B. The change of serum TGF-β1、Angiotensin II and urinary protein in 8th week after administration (following 12 wk after nephrectomy)

Groups	Dose (mg/kg)	TGF-β1 (ng/ml)	Change (%)	AII (pg/ml)	Change (%)	UP (mg/day)	Change (%)
Sham		20.1±6.2		54.5±22.7	12.7	18.3±2.5	
Model		46.33±14.74	130.5↑	94.5±7.4#	73.4↑	40.7±14.2#	122.5↑

Benazepril	4	40.9±26.6	11.72↓	74.3±13.2	21.4↓	51.1±23.6	25.8↑
Losartan	10	18.7±9.2	59.6↓	96.7±32.1	2.2↑	32.7±10.3	19.6↓
Compound	7.5	20.0±6.7	56.8↓	63.9±13.2*	32.4↓	30.1±16.6	26.0↓
149	15	18.6±12.2	59.9↓	49.9±21.3*	47.2↓	30.4±16.2	25.3↓
	30	18.9±10.1	59.2↓	41.0±12.5*	56.6↓	34.3±12. 1	15.7↓

Note: *P<0.05, compared with the model group; #P<0.05, compared with the sham group. ↑: increase; ↓: decrease

C. The change of serum creatinine (Scr.) and BUN concentration in 12th week after administration (following 16 weeks after nephrectomy)

Groups	Dose (mg/kg)	Scr. (mg/dL)	Change Rate (%)	BUN (mg/dL)	Change Rate(%)
Sham	-	2.25±0.39		21.24±3.354	
Model	-	2.71±0.49#		38.93±8.755#	83.32↑
Benazepril	4	2.28±0.70	20.70↑	39.48±7.109	1.41↑
Losartan	10	2.21±0.48*		37.84±5.672	2.80↓
Com. 149	7.5	2.73±0.78	19.01↑	39.42±4.686	1.25↑
	15	2.63±0.38		37.32±5.467	4.14↓
	30	2.10±0.71*	22.73↓	36.60±5.422	5.99↓
			0.75↓		
			2.87↓		
			28.82↓		

Note: *P<0.05, compared with the model group; #P<0.05, compared with the sham group; ↑: increase; ↓: decrease.

D. The change of serum TGF-β1、Angiotensin II and urinary protein in 12th week after administration (following 16 wk after nephrectomy)

Groups	Dose (mg/kg)	TGF-β1 (ng/ml)	Change (%)	AII (pg/ml)	Change (%)	UP (mg/day)	Change (%)
Sham		18.2±8.9		30.0±7.6		16.5±17.3	
Model		12.8±7.9		61.7±24.3	105.7↑	54.2±26.1#	228↑
Benazepril	4	12.8±14.8	0. 57↑	47.8±12.0	22.6↓	66.3±31.9	22.3↑
Losartan.	10	11.8±12.6	7.48↓	38.9±17.4*	37.2↑	39.3±14.2	18.4↓
Com. 149	7.5	13.6±7.1	6.28↑	48.3±48.5	21.6↓	66.7±38.8	23.1↑
	15	12.3±7.7	3.91↓	41.3±28.4	33.0↓	52.3±34.4	0.06↓
	30	11.6±6.7	9.38↓	19.2±9.19*	68.6↓	48.2±31.6	11.1↓

Note: *P<0.05, compared with model group; #P<0.05, compared with sham group. ↑: increase; ↓: decrease

E. Pathological results

wk	Groups	Glomerulosclerosis Grade (%)					
		0 Grade	I	II	III	IV	Total
8 wk afte ad.	Model	10.0±17.3	38.7±21.2	31.5±17.3	13.6±18.3	1.8±3.3	4.9±1.5
	Benazepril	12.2±19.0	29.6±23.9	28.1±18.1	19.6±24.2	5.9±11.2	5.5±2.6
	Losartan	54.4±29.2**		13.7±14.0	1.1±3.3**	0	2.8±1.0**
	Com.149		31.9±20.0	7.5±8.8*	17.5±30.7	6.7±13.4	
	30mg/kg	28.3±20.8		18.8±9.9	13.3±20.7	2.9±8.2	4.9±3.5
	15mg/kg	37.5±29.3*	34.2±23.0	30.0±20.8	23.2±28.5	8.0±13.0	3.9±1.7*
	7.5mg/kg	15.7±19.0					5.7±2.9

			27.5±16.3				
			24.0±21.1				
12	Model	0	3.3±6.4	29.0±23.4	50.5±18.7	17.6±14.1	8.5±1.3
wk	Benazepril	0	0	19.1±27.1	46.2±15.6	34.8±29.9	9.8±1.4
after	l	0	10.0±2.9	45.7±17.7	41.9±25.6		7.2±1.3**
r	Losartan					2.8±4.8	
ad.	Com.149	0	2.0±4.5	71.3±11.5**	26.7±7.8**		
	30mg/kg	0	8.1±14.1			0	6.7±0.2**
	15mg/kg			38.1±27.4	46.2±26.1	9.1±12.6	
							7.7±1.7
	7.5mg/kg	0	2.3±6.3	37.1±16.0	51.4±8.6	13.8±20.6	8.6±1.9

Note: *P<0.05, **P<0.01, compared with the model group.

The above various parameters with compound 149 treatment are all better than those of the Benazepril group and are equivalent to those of the Losartan group. Moreover, pathological results show that the test compound had no significant affection with the major organs such as heart, liver, spleen, and lungs.

Example 4. Inhibition of the test compound on kidney tubulointerstitial fibrosis caused by unilateral ureteral obstructed(UUO) in rats

Male Wistar rats with initial body weights of 180~230g were used. Unilateral ureteral obstruction was performed under pentobarbital anesthesia (35mg/kg) and sterile conditions. Via a midline incision, the

left ureteral was ligated. Sham surgery was performed by making a midline incision but leaving ureteral intact. Following surgery, rats were randomly divided into sham, model, Benazepril (4mg/kg/day) and Losartan (10mg/kg/day) as positive control, and compound 149 (5mg/kg/day, 10mg/kg/day and 20mg/kg/day). Starting 2 day before surgery, Benazepril, Losartan and compound 149 were administered for 16 days orally. BUN and creatinine in serum were determined in the 11^{th} and 16^{th} day (Table 6) following initiation of Benazepril, Losartan and compound 149 treatment, at which time animals were killed and the kidneys were harvested. Tissues were dissected, weighed, fixed in 10% formaldehyde and embedded in paraffin wax for pathological analysis. The 9^{th} day serum BUN and creatinine in model group after surgery increased 78.7% ($P<0.01$) and 20.73%($P<0.05$) respectively.

Tab. 6 Inhibition of test compound on kidney tubulointerstitial fibrosis caused by unilateral ureteral obstruction (UUO) in rats

Groups	Dose (mg/kg)	Scr. (mg/kg)	Change Rate (%)	BUN (mg/dL)	Change Rate (%)
Sham	--	1.45±0.44		16.23±2.70	
Model	--	2.20±0.14$^{\#}$	51.58↑	27.54±3.32$^{\#}$	69.73↑
Benazepril	4.0	1.92±0.29	12.50↓	20.99±1.58*	23.78↓
Losartan	10.0	2.15±0.51	2.31↓	23.88±2.94	13.30↓
Com.149	5.0	1.58±0.49*	28.24↓	23.71±4.17	13.92↓
	10.0	1.61±0.36*	26.50↓	20.76±1.56*	24.61↓
	20.0	1.60±0.14*	27.27↓	20.77±2.04*	24.58↓

Note: $^{*}P<0.05$, compared with model group; $^{\#}P<0.05$, compared with control group; ↑: increase, ↓: decrease.

In this assay, the improvement of each biochemical index with

compound 149 treatment are more significant than those of the Losartan group, and equivalent to those of the Benazepril group. There was slight different in the pathological changes: the inflammatory cell infiltration in the Benazepril group was more significant, and 4/7 of the animals had focal abscess formations in the medulla of kidney, many kidney cell necrosis and inflammatory cells and abscess cells overlapped in the Benazepril group. The inflammatory cell infiltration and tubulointerstitial fibrosis were significantly attenuated in both compound 149 and the Losartan groups. That is to say, compound 149 is better than Benazepril and equivalent with Losartan in the pathological results.

Example 5. Primary acute toxicity test for test compound

5g/kg and 10g/kg of compound 149 were administered orally to mice once and observed for 14 days. Body weights of 48 hours after administration in mice were no different. At 14^{th} day after administration, the mouse average body weights in 5g/kg and 10g/kg groups increased 7g and 5g respectively. There was no any other different for every animal and no death were observed..

Example 6. Ames test

His^- type *Salmonella typhimurium* TA97, TA98, TA100 and TA102 were employed. Concentration of the test compound was 0.5, 5.0, 50.0, 500.0, 5000.0 μg/plate. S9 was the microsome component of liver homogenate of a rat weighted 200g. The test compound 149 was tested in the presence or absence of S9.

According to the *Salmonella typhimurium*/mammalian microsome enzyme mutagenic test method revised by Ames (1983), metabolism activated or non-metabolism activated plate incorporation assay was conducted on compound 149, and the strain which passed the

assay was seeded to the culture medium and incubated at 37°C under shaking for 15 hours. 100μl compound solutions with various concentrations were added to 0.1 ml of the culture liquid, and then S9 mixture or phosphate buffer was added, and the mixtures were incubated in a 37°C water bath for 20 minutes. After that, 2ml of upper layer agar was added, mixed well and poured into a plate with lower layer agar and incubated at 37°C for 48 hours. The number of the colonies in each plate was counted.

The results show that the number of colony formation of Salmonella Typhimurium TA97, TA98, TA100 and TA102 induced by compound 149 did not increase. It suggests that compound 149 has no mutagenesis.